

Vitamin B₁₂
Selected Annotated Bibliography
1954



MERCK & CO., INC
Manufacturing Chemists
RAHWAY NEW JERSEY

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Forsword

This Selected Annotated Bibliography on Vitamin B₁₂ makes available the wealth of clinical and experimental data derived from the medical and related scientific literature from 1948 through the first half of 1953. Accordingly most of the abstracts originally published in the Annotated Bibliography on Vitamin B₁₂ (1950) have been incorporated into this present and greatly expanded volume.

In Part I the material has been arranged with reference to therapeutic use of vitamin B₁₂ in various systems or organs of the body and in certain physiologic and nutritional conditions and states—both from the clinical and the experimental standpoint. In Part II will be found analytical data pertaining to vitamin B₁₂ itself, its identification, characteristics and properties, and other salient aspects.

It is hoped that this comprehensive collection of abstracts and reference data on vitamin B₁₂ will prove useful to those engaged in basic and clinical research and in the practice of medicine.

MERCK & CO., INC.
CHEMICAL DIVISION
Rahway N J

MEDICAL DIVISION
January 1954

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Part I

Therapeutic Uses of Vitamin B₁₂

Blood and Lymphatic

ANEMIAS

MACROCYTIC ANEMIA

1. STONE, R. E., and SPIES, T. D. *The effect of liver extract and vitamin B₁₂ on the mucous membrane lesions of macrocytic anemia*, J. Lab. & Clin. Med. 33 1019-1023, Aug. 1948.

In the authors' experience, folic acid or thymine is without effect on very severe mucous membrane lesions of pernicious anemia, but liver extract produces prompt improvement which usually is maintained for one to nine months. Two patients who had a severe recurrence of such lesions have been treated with vitamin B₁₂, with at least temporary relief—they have been observed for two weeks to date. One case is reported in detail. In this case 15 mcg. was given intramuscularly. Within 24 hours there were equivocal signs of improvement. After 48 hours hyperemia of the tongue and buccal mucosa had definitely decreased. By the fifth day the color of the mucosa of the tongue and oral cavity appeared nearly normal, the external fissures were dry and apparently healing, and the tongue was less swollen. By the thirteenth day many fine papillae were visible over the entire upper surface of the tongue, and the external fissures were completely healed. All soreness and burning sensation of the mouth, tongue, and external fissures began to subside within 48 hours after the injection and completely disappeared within five days.

2. PATEL, J. C. *Crystalline anti-pernicious-anemia factor in treatment of two cases of tropical macrocytic anemia*, Brit. M. J. 2 934-935 Nov. 27 1948.

The effects of treatment with Lester Smith's crystalline anti-pernicious anemia factor in 2 cases of tropical macrocytic anemia are reported. Both patients received a single injection of a solution containing 80 mcg. of the factor. In each case there was a pronounced reticulocyte response, of 11.5 per cent on the sixth day in one patient, and of 86.8 per cent on the fifth day in the other. Each patient also showed an increase in red blood cells and hemoglobin and change of the bone marrow from megaloblastic to normoblastic. Improvement in general condition paralleled that which occurred in the blood picture, as evidenced by greatly increased appetite and rapid gain in weight. One patient, however, had a recurrence of stomatitis ten days after administration of the factor.

3. GOLDSMITH, G. *Vitamin B₁₂, pteroylglutamic acid and liver extract in the treatment of macrocytic anemia*, Am. J. Med. 7 258, Aug. 1949 (In Soc. Proc.)

Administration of vitamin B₁₂ to 6 patients and pteroylglutamic acid to 24 with macrocytic anemia in relapse was followed by an increase in reticulocytes, erythrocytes and hemoglobin. A normal or slightly subnormal blood picture was maintained in 12 patients for more than six months with 5 mg. of pteroylglutamic acid daily. Doses of 2.5 mg. a day were equally effective in 2 patients with sprue and 2 with nutritional macrocytic

anemia, but the blood count fell in 2 patients with pernicious anemia. Liver extract maintained the erythrocyte count and hemoglobin at higher levels than did 5 to 30 mg. of pteroylglutamic acid daily in 4 of 5 patients. Substitution of vitamin B₁₂ for pteroylglutamic acid in 1 patient was followed by improvement in the blood picture.

Of 9 patients with pernicious anemia treated with pteroylglutamic acid, 2 developed a neurologic relapse during therapy while existing neurologic changes in 2 others were unaffected. Neurologic abnormalities were reversed by vitamin B₁₂ in 1 patient who received this therapy.

4. BETHELL, F. H. *Treatment of macrocytic anemias with vitamin B₁₂*, J. Am. Dietet. A. 26 89-92, Feb. 1950.

At the Simpson Memorial Institute, 20 patients with Addisonian pernicious anemia have been treated with vitamin B₁₂ by intramuscular injection for periods up to 18 months. Satisfactory remissions have been maintained in each case. Recommended initial therapy for patients in relapse is 10 mcg. a day for the first week, 10 mcg. three times a week during the next two weeks, and twice a week thereafter until blood values are within normal limits. Maintenance therapy should be adjusted to the needs of the individual. If vitamin B₁₂ concentrates are used instead of the pure crystalline compound, their potency should be expressed in U.S.P. antianemia units.

The response of macrocytic anemias other than pernicious anemia to treatment with vitamin B₁₂ has been variable. The author has observed complete failure of response to the vitamin in one patient with macrocytic anemia of the puerperium who subsequently responded to folic acid therapy (J. Lab. & Clin. Med. 33 1477 1948), but other investigators have reported good results in tropical sprue associated with macrocytic anemia and in nutritional macrocytic anemia, and in some cases of nutritional macrocytic anemia in infancy.

As previously reported (Bethell et al. *Univ. Hosp. Bull., Ann Arbor* 15 49 1949) vitamin B₁₂ given orally alone has proved ineffective, but 5 mcg. a day in combination with extracts of hog duodenal mucosa (which have no hemopoietic activity when given alone) induce remissions. Six patients with previously untreated pernicious anemia have responded satisfactorily to this treatment.

*University of Michigan
Ann Arbor, Mich.*

5. GOLDSMITH, G. A. *Effects of vitamin B₁₂ given orally and parenterally and of a concentrate of "intrinsic factor" in macrocytic anemias*, Am. J. Med. 9 399 Sept. 1950 (In Soc. Proc.)

Twenty-two patients with macrocytic anemia have been treated with oral or parenteral vitamin B₁₂ alone or with folic acid or a concentrate of "intrinsic factor."

In 200 mg. this substance markedly potentiated the effect of B₁₂ in pernicious anemia patients.

The isolation and synthesis of folic acid disclosed another substance necessary for normal hematopoiesis. Severe macrocytic anemia may develop in the absence of folic acid, but folic acid, though it causes initial remission in pernicious anemia, does not prevent neurologic degenerations or relapse in this disease. Since liver extract will arrest the neurologic disturbances, it is evident that folic acid cannot replace liver factor nor is the function of liver factor merely to make folic acid available.

How these erythropoietic substances affect cell metabolism is not well known, but there is considerable evidence that they function as enzymes in the synthesis of nucleic acid. Probably folic acid furnishes the substrate for the formation of folinic acid, or the citrovorum factor instead of being active itself. Citrovorum factor appears to facilitate formation of pyrimidines from amino acids and other simple materials, while B₁₂ seems to be active in the synthesis of nucleosides from pyrimidines and purines. Theoretically citrovorum factor should be an ideal antidote for aminopterin poisoning, because it counteracts the B₁₂ antagonism of aminopterin.

Vitamin B₁₂ is effective parenterally in very small doses, 10 mcg. per day being a generous optimum dose during the first week of pernicious anemia therapy. Given orally B₁₂ is not effective unless combined with intrinsic factor and ten times the parenteral dose is necessary. Folic acid administered alone in pernicious anemia is dangerous because it does not prevent neurologic manifestations. Folic acid may be very effective, however, in macrocytic anemias caused by dietary insufficiency, pregnancy, sprue, and other disturbances of intestinal absorption, in which neurologic degeneration seldom occurs.

There are 19 references.

11. HERRIGAN, D. L., and HEINLE, R. W. *Refractory macrocytic anemia with defect in vitamin B₁₂ binding and with response to normal plasma*, J. Lab. & Clin. Med. 40 811-812, Nov 1952 (in Soc. Proc.)

"In vivo studies in this laboratory and in vitro bone marrow culture studies by others have suggested that vitamin B₁₂ is utilized for hematopoiesis in a bound form. It was postulated, therefore, that a deficiency in binding of the vitamin might result in refractory macrocytic anemia.

"A patient was observed who has had unexplained anemia for eighteen years which did not respond to crude liver extract given intramuscularly every week nor to iron orally. Free HCl was present in fasting gastric contents. Study of hematologic remissions and relapses in this patient during the past year have provided data relative to the role of B₁₂ binding in the utilization of the vitamin for hematopoiesis. Hematologic findings during relapse included macrocytic anemia, reticulocytosis, and normoblastic bone marrow hyperplasia. Remissions have been produced by a single intramuscular dose of 90 µg. of vitamin B₁₂ or by the intravenous administration of 250 ml. of human plasma. Subsequent to administration of either

there was a rise of hemoglobin from 9 to 12 G 100 cc. of blood, and of the hematocrit from 31 to per cent. In either instance, remission was not maintained with vitamin B₁₂, 30 µg. intramuscularly every two weeks, or with oral folic acid, 10 mg. daily.

"Intramuscular administration of 0.5 µg. of Co⁵⁷ labeled vitamin B₁₂ resulted in the urinary excretion of 60 per cent of the administered dose within twenty four hours. (Normal persons excreted 10 to 10 per cent under similar conditions.) After giving normal plasma, the urinary excretion of the same dose of the labeled vitamin decreased to 24 per cent. Uric acid in the erythrocytes was elevated during relapse and decreased to normal with remission. No evidence of increased blood destruction was demonstrated.

"These observations suggest that (a) the anemia in this subject results from B₁₂ deficiency caused by failure of proper binding of the vitamin, and (b) normal plasma contains a factor which corrects this defect. This factor may be (a) binding substance itself, (b) a substance necessary for the combination of B₁₂ and the binding substance endogenously or (c) amounts of the vitamin in bound form sufficient to promote hematopoiesis."

Cleveland, Ohio

12. BEYERS, M. R., DIEFENBACH, W. C. L., MARK, H., and MEYER, L. M.: *Interrelationship of folic acid and vitamin B₁₂ in macrocytic anemia associated with linitis plastica*, Acta med. scandinav. 142 351 Nov 5 1952.

13. McKUSICK, V. A. *Boeck's sarcoid of the stomach with comments on the etiology of regional enteritis*, Gastroenterology 23 103-113, Jan. 1953.

Two cases of Boeck's sarcoid of the stomach are described. One of the patients also had macrocytic anemia which responded dramatically to parenteral administration of vitamin B₁₂.

John H. Hays Hospital
Baltimore, Md.

MEGALOBLASTIC ANEMIA

14. UNGLEY, C. C.: *Modern management of megaloblastic anemias* Lancet 2 66, July 10 1948. Brit. Med. J 2 154, July 17 1948.

15. DAVIDSON, S. *Folic acid and its conjugates in the pathogenesis of megaloblastic anaemia*, Lancet 2 814-815, Oct. 29 1949 (in Letters to the Editor)

The writer examines the data presented by Wilkinson and Israels (Oct. 15) from which they concluded that patients with Addisonian pernicious anemia have a normal capacity to release folic acid from its conjugates and that folic acid or its conjugates play no major part in the etiology of the human pernicious anemia syndrome. He finds these deductions unwarranted, and believes that present knowledge indicates a requirement of both folic acid and vitamin B₁₂ for prevention of megaloblastic blood formation.

Edinburgh
Edinburgh, Scotland

16. MUELLER, J. F., HAWKINS, V. R., and VILTER, R. W. *Liver extract refractory megaloblastic anemia*, Blood 4 1117-1123, Oct. 1949

This is a case study of a patient with macrocytic anemia, megaloblastic maturation arrest in the bone marrow, glossitis, hyperreflexia and diminished vibration perception in the feet. Vitamin B₁₂ given parenterally (8 mcg. initially and on each of three successive days) and liver extract produced no improvement in any respect. Folic acid, however, in a dosage of 7.5 mg. a day intramuscularly for 10 days, had a favorable effect on all the abnormalities except the neurologic signs. The patient appeared to have the type of megaloblastic anemia that is described as "achrestic anemia" and "refractory megaloblastic anemia." It resembles "Wills factor deficiency anemia" and some cases of pernicious anemia of pregnancy. Folic acid deficiency apparently was not the primary etiologic factor since the urinary excretion of folic acid was within normal limits. Deficiency of an unknown factor probably the equivalent of the "Wills factor" is suggested. It seems likely that folic acid overcomes deficiency of this factor by a "mass action," which may also explain its hemopoietic action in pernicious anemia. A later hematologic response to 10 thymine which occurred when the case described relapsed, is consistent with such a theory.

17. SCHBIATOLLA, E., GIBSON, A., and CARLISLE, J. M.: *Crystalline vitamin B₁₂ in the treatment of megaloblastic anemias*, Postgrad. Med. 6 803-310 Oct. 1949 Mod. Med., Oct. 1 1949 pp. 60-64.

This article is adapted from the Scientific Exhibit presented by members of the Medical Division of Merck & Co., Inc., at the 1949 convention of the American Medical Association at Atlantic City. Crystalline vitamin B₁₂, which was first isolated in the Merck laboratories early in 1948, is depicted in color. This is a pure anti-anemia substance of high potency in the treatment of anemias characterized by erythrocyte maturation arrest. Vitamin B₁₂ has also been shown to be an essential "growth factor" for certain species of animals.

The pathogenesis of the megaloblastic anemias and the diagnostic criteria for pernicious anemia are shown pictorially. The bone marrow and blood responses to vitamin B₁₂ therapy are illustrated in color while a chart shows the improvement in neurologic complications obtained by this treatment.

18. MILLER, C. M., and MOORHOUSE, E. H. *Treatment of megaloblastic anemia with vitamin B₁₂*, Brit. M. J. 2 1511-1512, Dec. 31, 1949

A satisfactory clinical and hematologic response was obtained in each of 2 cases of megaloblastic anemia with vitamin B₁₂ prepared from Streptomyces (Glaxo Laboratories). The patients received 20 mcg. initially by intramuscular injection and the same amount at each of a series of injections several days later the total dosages being 100 and 140 mcg. Continued treatment was impossible because of the limited supply of B₁₂.

19. UNGLEY C. C. *Vitamin B₁₂ in megaloblastic anemias*, Lancet 1 930, May 13, 1950 (in Letters to the Editor)

The letter reads: "In referring to the effect of vitamin B₁₂ in megaloblastic anemias without gastric atrophy Dr. Tuck and Dr. Wittaker (April 22, p. 757) incorrectly abbreviated a report of my remarks at the British Association meeting last year which was already too condensed to be accurate. These anemias are a 'mixed bag' and the response to vitamin B₁₂ varies widely. It is true that megaloblastic anemias of pregnancy and the puerperium in temperate climates did not respond, but Patel and Kocher report good results in a tropical variety of the syndrome.

"Megaloblastic anemias associated with intestinal disorders—even those encountered in this country—have not always failed to respond to vitamin B₁₂. On the other hand, a state refractory to vitamin B₁₂ may exist temporarily even in a patient presumed to have gastric atrophy. Such cases are rare. In one instance the anemia became responsive to vitamin B₁₂ only after small amounts of folic acid had been supplied. This recalls the pigs of Hietala, Welch, and Pritchard, which were so depleted of hemopoietic factors that folic acid had to be given before liver extract would work, and vice versa."

Royal Victoria Infirmary
Tromsø, Spitsbergen, England

20. UNGLEY C. C. *Vitamin B₁₂ and other dietary factors in megaloblastic anemias*, Brit. J. Nutrition 4 252-259 1950.

21. ROYAL SOCIETY OF MEDICINE: *Pathogenesis and treatment of megaloblastic anemias*, Brit. M. J. 1 1367 June 10, 1950 (In Soc. Proc.)

22. FOY H., KONDI, A., and HARGREAVES, A.: *The megaloblastic anemias*, Lancet 1 1172-1173, June 24 1950 (in Letters to the Editor)

This communication is a discussion of the nature and etiology of the megaloblastic anemias. The writers believe that the megaloblastic macrocytic anemias have a different etiology from the megaloblastic normocytic anemias and believe the presence or absence of the giant stab cells to be significant. They cite the responses of the several types of anemia to folic acid and vitamin B₁₂ and try to relate these differences to their discussion.

Karachi, Bombay
India

23. PATEL, J. C. *Vitamin B₁₂*, Lancet 2 407 Sept. 23, 1950 (In Soc. Proc.)

"Dr. J. C. Patel (India) had found among patients with tropical megaloblastic anemia that those with free HCl in the gastric juice often respond to B₁₂ orally in doses of 20-600 µg. daily some of the achlorhydrics showed an irregular response, but in true pernicious anemia there was no response at all."

24. UNGLEY C. C. *Vitamin B₁₂. Part 2. A review of the clinical aspects* Nutrition Abstr. & Rev. 21:1-26, July 1951 (Abstr. J. Am. Dietet. A. 23 160, Feb. 1952)

"This review of the clinical aspects of vitamin B₁₂ is concerned primarily with the effects of oral and parenteral administration of the vitamin to human subjects suffering from megaloblastic anaemias. Current views of the role of Castle's intrinsic factor in the absorption and utilization of the vitamin are presented also, along with a discussion of its fate after oral and parenteral administration."

25 GIRDWOOD R. H.: *Vitamin B₁₂ and folic acid in the megaloblastic anaemias* Edinburgh M J 58 309-335, July 1951.

26. WITTS, L. J. *Pathogenesis of the megaloblastic anaemias*, Lancet 2 367-369 Sept. 1 1951.

27 UNGLEY C. C.: *Megaloblastic anaemias*, Lancet 2 1068, Dec. 8, 1951 (in Soc. Proc.)

In discussion at a meeting of the Royal Society of Medicine, Dr C. C. Ungley suggested that the gastric atrophy that was the classical feature of pernicious anaemia led not only to a nutritional defect and loss of vitamin B₁₂, but also, by changes in the bacterial flora of the upper intestine, to the formation of 'toxic factors'. With the help of diagrams he showed how these two factors could produce the known syndromes of megaloblastic anaemia, but he gave warning that these diagrams were as yet theoretical and that the experimental basis for many of the reactions shown was still missing or unconfirmed. Dr Ungley believed that both folic acid and vitamin B₁₂ were concerned in normal erythropoiesis: folic acid accelerated the utilization of vitamin B₁₂, so that in pernicious anaemia the action of folic acid fell off when remaining stores of vitamin B₁₂ were exhausted. He quoted experiments showing that small doses of vitamin B₁₂ would act when given orally only if it was previously treated with normal gastric juices. It had been suggested that gastric juice acted by preventing the bacteria in the intestine from metabolising vitamin B₁₂, so that when there was achylia B₁₂ in the food was taken up by the bacteria and did not reach the erythropoietic tissues. Dr Ungley 'sterilised' the intestinal tract of a patient by giving Aureomycin, and he found that 80 µg. of vitamin B₁₂ given orally was still ineffective, though after incubation with normal gastric juices 80 µg. produced a good response as before. He believed, therefore, that the 'intrinsic factor' in gastric juice actually combined with vitamin B₁₂ to form a new factor that could pass the intestinal mucosa, but there was evidence that the combination was split up again and the vitamin B₁₂ freed in the body fluids after absorption. There seemed to be a logarithmic relation between the parenteral dose of vitamin B₁₂ and the rise of the red-cell count in the first 15 days after administration of up to 320 µg.; bigger doses gave less than the expected response."

28. GIRDWOOD R. H.: *The interrelationships of factors that influence the megaloblastic anaemias* Blood 7 77-93 Jan. 1952.

Author's conclusions after reviewing 112 references. "This review of a vast literature is necessarily incomplete, and it is possible that investigations that may eventually prove to be of great importance have been omitted. The work reviewed here indicates that vitamin B₁₂, pteroylglu-

tamic acid and the citrovorum factor are probably concerned with the metabolism of the maturing red quite apart from their other undoubted metabolic which have not been considered. Pteroylglutamic acid and vitamin B₁₂ play a part in methyl metabolism and the metabolism of glycine, serine, ethanolamine and formic acid, all of which are probably concerned both with the formation of purines and pyrimidines and also with the synthesis of porphyrins. This, too may be significant in relation to blood metabolism.

"Bone marrow cultures have suggested that substances may exist in the serum that inhibit normal blood formation. If this is true of what takes place in the body we do not know the mode of formation of such inhibitors, or whether any part of the action of the antimetabolite factors is concerned with the overcoming of such inhibitors.

"Two main theories about the interrelationships of these factors have been considered. The view that vitamin B₁₂ is concerned with the release of pteroylglutamic acid from its conjugates is unsatisfactory mainly because it does not explain why pernicious anaemia patients cannot be maintained indefinitely in hematologic remission with synthetic pteroylglutamic acid. It must be admitted, however, that there still exists the possibility that one of the actions of vitamin B₁₂ is to do this.

"The other theory put forward in part by Wright, Skeggs and Huff and elaborated by Viller et al. is based very largely upon bacteriologic findings and requires further investigation in relation to megaloblastic anaemias in man. According to this theory pteroylglutamic acid which may be temporarily effective in a dosage of as little as 1 mg. daily acts by a 'mass action' effect in pernicious anaemia. Several workers have suggested that if it does act in such a way traces of vitamin B₁₂ in the body may be used up in the process. There is some evidence that vitamin B₁₂ may not entirely be lacking from the tissues in pernicious anaemia. Subacute combined degeneration of the cord, the pathogenesis of which is uncertain, might then arise when vitamin B₁₂ depletion was complete or almost complete.

"Finally there is evidence for the presence of numerous forms of vitamin B₁₂ in the body and to the complexities of the interrelationships of the various factors there is added the complexities of the metabolic interrelationships of the various forms of vitamin B₁₂."

University of Edinburgh
Edinburgh, Scotland

29 HARRISON H. E., in discussion on May C. D., Hamilton, A., and Stewart, C. T.: *Folic acid deficiency experimental and clinical*, A.M.A. Am. J. Dis. Child. 84 475-477 Oct. 1952 (in Soc. Proc.)

"I have observed a baby with megaloblastosis who apparently had a congenital deficiency of absorption of vitamin B₁₂. This child had a characteristic picture of megaloblastic anaemia but responded not at all to folic acid, even in extremely large doses or in doses given intramuscularly but did respond in typical fashion to vitamin B₁₂ given intramuscularly and to very large doses given orally. The child had a relapse every time administration of vitamin B₁₂ was stopped."

0. UNGLEY C. C. *The pathogenesis of megaloblastic anemias and the value of vitamin B₁₂*. Brit. J. Nutrition 6 299-315 (No. 3) 1952.

1. THEDERING F., and RIETHAUER: *Vitamin B₁₂ and folic acid in the treatment of megaloblastic anemia*. Folia clin. internat. 2 371 1952 (abstr. Semana med. 102 96, Jan. 15 1953).

The authors recommend combined therapy of vitamin B₁₂ and folic acid for pernicious type anemia. If it is classic type pernicious anemia, vitamin B₁₂ alone or in combined therapy is effective, but in some megaloblastic anemias, combined therapy has been found more effective. The original article is described as containing a review of the literature and the authors' clinical experience.

32. GIRDWOOD R. H. *The relationships between vitamin B₁₂, folic acid and folinic acid*. Brit. J. Nutrition 6 315-324 (No. 3) 1952 (abstr. J. Am. Dietet. A. 29 160, Feb. 1953).

"Experiments indicate the importance of supplying both vitamin B₁₂ and folic acid to prevent megaloblastic anemia in the pig. Protein deficiency increases the need for folic acid. Monkeys placed on a low-protein diet have been found to develop megaloblastic anemia more rapidly if there were also an ascorbic acid deficiency. When anemia did develop folic acid was rapidly effective in treatment. Ascorbic acid was more slowly effective and vitamin B₁₂ was ineffective, but B₁₂ plus ascorbic acid gave a prompt response. The normal daily intake of folic or folinic acid required to prevent nutritional megaloblastic anemia in man is uncertain. All that can be concluded at present is that vitamin B₁₂ and folic acid both appear to be required for normoblastic blood formation, and that ascorbic acid deficiency appears to be related to the development of megaloblastic anemia."

PERNICIOUS ANEMIA

33. WEST R.: *Activity of vitamin B₁₂ in Addisonian pernicious anemia*. Science 107 398, April 16, 1948.

Vitamin B₁₂, the new crystalline compound isolated from liver has produced positive hematologic responses in 3 patients with pernicious anemia following single intramuscular injections of 3, 6, and 150 mcg., respectively. Four similar patients given single injections of amorphous liver concentrates containing 20,000 to 40,000 LLD units of the compound showed strong or maximal hematologic responses, while 3 patients given concentrates containing only 10,000 LLD units or less manifested little or no response. In the 3 patients given crystalline vitamin B₁₂, there has been a rise in reticulocytes, red cell count, and hemoglobin, but it is still too early to say whether the blood picture will return to normal without further treatment. In one of these patients there has been a striking rise in the white blood cell count, the platelets have risen from 120,000 to 340,000. It is of interest that Preparation No. 31 of Dakin, Ungley and West (*J. Biol. Chem.* 115 771, 1935) which was clinically active, has been shown by microbiologic assay to contain about 30,000 LLD units (3 mcg.) of vitamin B₁₂ per 25 mg., which is the dose used at the time.

34. STOKSTAD E. L. R., PAGE, A., JR., PIERCE, J., FRANKLIN A. L., JUKES, T. H., HEINLE, R. W., EPSTEIN, M., and WELCH, A. D. *Activity of microbial animal protein factor concentrates in pernicious anemia*. J. Lab. & Clin. Med. 13 860-864, July 1948.

A nonmotile, rod-shaped organism from hen feces has been found to produce an animal protein factor which is necessary for chick growth and which is capable of producing hematologic and clinical responses in pernicious anemia. Two concentrates of the growth media, one prepared by clarification and precipitation with ammonium sulfate (concentrate I) and the other by clarification alone (concentrate II) have been tested clinically. Concentrate I on chick assay had between 50 and 100 per cent the activity of a liver extract containing 10 anti-pernicious anemia units per cc., and on *Streptococcus faecalis* assay contained only 0.5 mcg. of folic acid per cc. Concentrate II had between 25 and 40 per cent the activity of a 10-unit liver extract and contained about 0.02 mcg. of folic acid per cc. Treatment with "conjugate" did not increase the folic acid value of either concentrate.

Concentrate I was given in daily intramuscular doses of 1 cc. for nine days to a patient 90 years of age suffering from pernicious anemia, with severe nausea and fecal vomiting. After the third injection nausea and vomiting ceased and did not reappear. The reticulocyte response was less prompt than the usual response to effective intramuscular liver therapy but the level reached was nearly maximal. The rise began on the fourth day and reached a peak of 20.6 per cent on the tenth. This peak was followed by a prompt increase in erythrocytes, leukocytes, and platelets, and in hemoglobin, with corresponding clinical improvement. After an interval of nine days a course of liver therapy was given, 1 cc. of a 10-unit extract being administered intramuscularly each day for nine consecutive days. A second, small reticulocyte peak, 6 per cent, occurred on the sixth day. The patient continued to improve.

Concentrate II was given to another aged patient with pernicious anemia. The dosage was the same as with concentrate I in the previous case, but treatment was continued for thirty days. The reticulocyte response was subnormal, a peak of 9.7 per cent occurring on the seventh day. There was a prompt increase in erythrocytes and leukocytes and in hemoglobin. After thirty days, the erythrocyte count had risen by more than 1,500,000 cells per cu. mm. and the hemoglobin from 7.7 to 10.8 Gm. per 100 cc. The mean corpuscular volume and mean corpuscular hemoglobin showed a decrease.

Whether this new factor is identical with the anti-pernicious anemia factor in liver extracts or with the recently isolated vitamin B₁₂ which is active in pernicious anemia has not yet been determined.

35. ADDINALL, C. R. *Recent advances in the chemistry of natural products: the isolation and properties of vitamin B₁₂*. Merck Report 57 4-7 Oct. 1948.

The author traces the course of therapy for pernicious anemia from liver substance, through liver extract, folic acid, and thymine to its present culmination in vitamin B₁₂.

36. BETHELL, F. H., in discussion on Haden, R. L., and Bortz, D. W. *Treatment of idiopathic pernicious anemia*, J.A.M.A. 138: 870-873 Nov. 20, 1918.

Dr Bethell discusses an article in which complete liver extract is described as the treatment of choice in pernicious anemia. He states that vitamin B₁₂ may lead to complete reversal of the specific treatment of pernicious anemia and related anemias. He has used vitamin B₁₂ and it has induced optimal hemopoietic responses in patients with pernicious anemia, even when given in doses as small as 1 mg. [1 microgram?] daily. The number of patients receiving the vitamin is not stated.

37. HALL, B. E., and CAMPBELL, D. C.: *Vitamin B₁₂ therapy in pernicious anemia. I. Effect on hematopoietic system. preliminary report*, Proc. Staff Meet., Mayo Clin. 23: 584-590, Dec. 8, 1918.

The effect on the hemopoietic system of vitamin B₁₂ given intramuscularly was studied in 11 patients with pernicious anemia in relapse. The reticulocyte response and the rate of rise in erythrocyte levels were comparable to those obtained when liver is employed. The reticulocyte peak occurred on the fourth to the seventh day after institution of therapy. In some cases the erythrocyte count rose to normal in five to seven weeks and in others it leveled off after it had exceeded 3,000,000/cu. mm. of blood and did not rise farther for one to three weeks.

The effect of approximately 1 microgram of vitamin B₁₂ was equivalent to that of 1 U.S.P. unit of extracts of liver or stomach mucosa. Various dosages were employed. In some cases 1 mcg. a day induced maximal hemopoietic responses. Weekly doses of 25 mcg. gave excellent hemopoietic responses in 3 patients, but in 2 of them the erythrocyte count ceased to rise when the interval between injections was prolonged beyond 12 days. A satisfactory response was noted in 1 case from intra-muscular administration of 5 mcg. of vitamin B₁₂ for five days and then of 5 mcg. three times a week. Maintenance doses have not yet been established.

Serial aspirations of the sternal marrow have shown regeneration of erythrocytes from megaloblastic to normoblastic types of cells in 48 to 72 hours after administration of relatively large doses of vitamin B₁₂. In one case normoblastic regeneration was virtually complete 48 hours after the administration of a single injection of 25 mcg.

38. HALL, B. E., and CAMPBELL, D. C.: *Effect of vitamin B₁₂ on the hematopoietic and nervous systems in Addisonian pernicious anemia*, J. Lab. & Clin. Med. 33: 1646, Dec. 1948 (In Soc. Proc.)

Six patients with pernicious anemia in relapse, 4 of whom had not received prior treatment, were treated with vitamin B₁₂. Doses of 25 mcg. given intramuscularly at weekly intervals produced excellent hematologic responses. When the interval between injections was more than a week the rise in erythrocytes was not always so great. Reticulocyte peaks were noted four to seven days after initiation of vitamin B₁₂ therapy and normal erythrocyte counts were reached in four to six weeks. Complete conversion of the bone marrow from megaloblastic to normoblastic regeneration occurred 48 to 90 hours after administration of vitamin B₁₂.

Glossitis was relieved by vitamin B₁₂ in the 4 cases in which it was present. Four of the 6 patients had sub-acute combined degeneration of the spinal cord. There was remarkable improvement in one of these patients who received 75 mcg. of vitamin B₁₂ over a period of 33 days but another showed no subjective or objective improvement after receiving 100 mcg. over a period of 62 days; the remaining 2 are still under observation. The necessity for observation over many months to determine whether vitamin B₁₂ will permanently protect the central and peripheral nervous systems in pernicious anemia is stressed.

39. CLOUGH, P. W.: *Further refinement of liver extracts effective in pernicious anemia*, Ann. Int. Med. 29: 1169-1172, Dec. 1948 (Editorial)

40. QUERIES AND MINOR NOTES: *Vitamin B₁₂ for pernicious anemia*, J.A.M.A. 142: 297 Jan. 28, 1950.

The editor is asked whether vitamin B₁₂ therapy entirely eliminates the need for liver therapy in patients with pernicious anemia. In reply it is stated that according to present indications, vitamin B₁₂ is effective in controlling both the blood and neurologic changes in patients with pernicious anemia. It is not known whether liver extract has any additional properties or not. It would appear to be safe to use vitamin B₁₂ injections without liver extract if proper blood and neurologic studies are made at intervals to determine whether there are any deleterious changes. A dosage of 10 mcg. of vitamin B₁₂ per week is recommended.

41. SPIES, T. D., STONE, R. E., KOCH, M. B., GRANT, H. M., and MOORE, M. M.: *The hemopoietic response of patients with pernicious anemia to crystalline vitamin B₁₂*, South. M. J. 43: 50-51, Jan. 1950.

Four patients with acute pernicious anemia in relapse showed a satisfactory hemopoietic and clinical response to vitamin B₁₂. One case history is reported: this patient responded to a single intramuscular dose of 10 mcg. of B₁₂. The reticulocytes reached a peak of 16 per cent on the tenth day after therapy and subsequently there was an increase in red blood cells and hemoglobin. Clinical response preceded the hemopoietic response.

*Northwestern University Medical School
Chicago, Ill.
William Hospital
Birmingham, Ala.*

42. BERGER, M.: *Vitamin B₁₂ in Addisonian pernicious anemia in a liver sensitive person*, New York State J. Med. 50: 331-332, Feb. 1, 1950.

A woman with Addisonian pernicious anemia developed sensitivity to liver. Folic acid controlled the hematologic situation, but symptoms of combined system disease developed. Treatment was changed to 10 mcg. of vitamin B₁₂ once a week, and improvement in the neurologic symptoms was evident within five days. After six weeks of treatment there was no subjective or objective evidence of combined system disease. At this point the supply of vitamin B₁₂ was exhausted, and it was necessary to substitute folic acid treatment. Neurologic symptoms returned under this treatment. Vitamin B₁₂ is particularly valuable in that it apparently contains factors effective against both anemia and combined system sclerosis.

An addendum states that since these observations were made another investigator has reported that vitamin B₁₂ is not efficacious in the treatment of the megaloblastic anemias of infants unless minute amounts of folic acid are present. This suggests that folic acid plays the role of a catalyst in this disease.

*Lancet Medical Department and Surgeon General
Toronto, N. Y.*

- 43 CHALMERS, J. N. M.: *Recent work on vitamin B₁₂*. *Lancet* 1 354 Feb. 25, 1950 (in Soc. Proc.)

In a discussion of vitamin B₁₂ in the anemias Dr Chalmers reported that "in his clinic a maintenance dose of 20-40 µg. fortnightly had mostly proved satisfactory in a few cases 60 µg. had been needed. In his experience vitamin B₁₂ did everything that liver did and it had proved useful in patients who had relapsed on unassisted liver extract. Eight cases treated with the unnamed substance were doing well. Vitamin B₁₂ was satisfactory in 4 patients allergic to liver extract 1 of these who suffered from crippling subacute combined degeneration, was restored to actively earning a living."

- 44 KINNEAR, T., and HUNTER, R. B.: *Therapeutic potency of vitamin B₁₂ derived from Streptomyces griseus culture liquor*, Edinburgh M. J. 57 65-71, Feb. 1950 (abstr. J.A.M.A. 143 1288-1289 Aug. 5, 1950)

The authors treated 34 pernicious anemia patients with vitamin B₁₂ obtained from *Streptomyces griseus* liquor. The authors believe that vitamin B₁₂ derived from *Streptomyces griseus* is satisfactory for treating pernicious anemia and for maintenance therapy. There is as yet no proof that vitamin B₁₂ will prevent the onset of subacute combined degeneration, but the evidence so far suggests that this condition does not progress when therapy is adequate, and in no case has it developed during treatment.

45. LEADING ARTICLE: *Vitamin B₁₂ and pernicious anemia*, *Lancet* 1 500-501, March 18, 1950.

It is stated that vitamin B₁₂ is effective in producing and maintaining remission in pernicious anemia. Several hypotheses concerning the etiology of pernicious anemia are discussed, and evidence is cited to support or disprove them. It is concluded that any practical hypothesis concerning the etiology of pernicious anemia must take into account the invariable achlorhydria and failure of secretion of the gastric enzymes, including intrinsic factor. Vitamin B₁₂ has properties resembling known extrinsic factors; however it is probably not the only source thereof.

46. HARTLEY F.: *Vitamin B₁₂ and pernicious anemia*, *Lancet* 1 594, March 25, 1950 (in Letters to the Editor)

The writer believes that the following statement from a leading article in the March 18 *Lancet* (Abstr. 45)) needs qualification. "There is now sufficient evidence for us to say that preparations of vitamin B₁₂ from liver or *Streptomyces griseus* will produce a remission in pernicious anemia, and that regular dosage will maintain

a normal blood picture." He states that in many cases vitamin B₁₂ is ineffective.

*The British Drug Houses, Ltd.
London, England*

47. UNCLEY C. C.: *Vitamin B₁₂ and pernicious anemia*, *Lancet* 1 593-594, March 25, 1950 (in Letters to the Editor)

The writer feels that certain references to his work in a leading article in the March 18 *Lancet* (Abstr. 45) require qualification. He states that 10 mcg. doses of vitamin B₁₂ given every two weeks, are adequate in some, but not in all, cases of pernicious anemia in producing satisfactory increases in red cells or hemoglobin. Those patients whose blood is examined only once in six months are usually given 80 to 60 mcg. every three weeks. Larger doses are given to patients with subacute combined degeneration. The writer further states that a mixture of 5 mcg. of vitamin B₁₂ with 50 cc. of human gastric juice given daily has approximately the same hemopoietic effect as a single dose of 10 mcg. of vitamin B₁₂ given parenterally. This relationship varies appreciably from case to case.

*Royal Victoria Infirmary
Hemel Hempstead, Herts. England*

48. GIRDWOOD R. H.: *Vitamin B₁₂ and pernicious anemia*, *Lancet* 1 594, March 25, 1950 (in Letters to the Editor)

In none of 8 patients with pernicious anemia in relapse nor in 2 controls were there any appreciable amounts of vitamin B₁₂ or folic acid in the small intestine. In these 5 subjects a day's stools yielded about 5 mcg. of vitamin B₁₂ and 0.5 mg. of folic acid. In a patient with ulcerative colitis, each cubic centimeter of material obtained from the lower end of the ileum contained about 0.03 mcg. of growth factors for *L. leichmannii* and 12 mcg. of folic acid. It was considered improbable that relatives and remissions in pernicious anemia could be related to changes in the flora of the small intestine.

*University of Edinburgh
Edinburgh, Scotland*

49. WATSON, J., LICHTMAN, H., GINSBERG, V., PIERCE, J. V., STOKSTAD, E. L. R., and JUKES, T. H.: *Use of vitamin B₁₂ in pernicious anemia*, *Am. J. Med.* 11 398, March 1950 (in Soc. Proc.)

A solution of crystalline vitamin B₁₂, 10 mcg. per cc., was prepared in 0.9 per cent sodium chloride and sterilized by filtration. This material was administered to 4 patients with Addisonian pernicious anemia. Of 2 who were given 1 mcg. daily intramuscularly one responded with a reticulocyte peak and a red cell rise higher than expected, and the other gave a submaximal reticulocyte response with a maximal red cell rise at three weeks. Hematologic response was satisfactory in the remaining 2 patients, who were given 1.5 and 2 mcg. daily by injection, respectively. Clinical improvement, including neurologic, was excellent in all 4 patients. It is concluded that 1 to 2 mcg. of vitamin B₁₂ daily by intramuscular injection is hemopoietically equivalent to one U.S.P. unit of liver extract and is comparable in potency to vitamin B₁₂.

Another patient who had pernicious anemia of pregnancy failed to respond to large amounts of liver extract, but failed to respond to 1 mcg. of vitamin B₁₂ daily but showed a dramatic response to the subsequent daily injection of 15 mg. of folic acid.

The oral administration of vitamin B₁₂ is being studied.

Kings County Hospital
Brooklyn, N. Y.
Laboratory
East River, N. Y.

50. MOLLIN D. L.: *Vitamin B₁₂ and pernicious anemia*, Lancet 1 690, April 8, 1950 (in Letters to the Editor)

The writer reports the treatment with vitamin B₁₂ of 19 succombing patients suffering from pernicious anemia. Remissions obtained were similar to those with the best liver extracts.

London, England

51. ERF L. A., and WIMER, B. M.: *Pernicious anemia: discussion of treatment with special reference to vitamin B₁₂*, GP 1 59-66, April 1950.

52. MEYER, L. M., SAWITSKY A., COHEN B. S., KRIM, M., FADEM, R., and RITZ, N. D.: *Oral treatment of pernicious anemia with vitamin B₁₂*, Bull. New York Acad. Med. 26 263-264, April 1950 (in Soc. Proc.)

Five patients with pernicious anemia in relapse were treated with daily oral doses of 75 to 150 mcg. of vitamin B₁₂ for periods up to seven months. Reticulocytosis was submaximal but clinical and hematologic response was satisfactory and a patient with evidence of combined system disease showed improvement in neurologic status. Parenteral administration of vitamin B₁₂ was effective in 2 patients who had not responded to daily oral dosage of 150 and 250 mcg., respectively. Two patients had a sub-optimal reticulocyte response, but satisfactory hematologic response and clinical improvement followed administration of 2 Gm. of desiccated hog duodenum mucosa and 10 mcg. of vitamin B₁₂ daily for two months. Two additional patients were given daily oral doses of 1.67 mg. of folic acid and 25 mcg. of vitamin B₁₂ for five weeks. Reticulocytosis was maximal and the rise in hemoglobin and erythrocytes was rapid. Increase of leukocytes above 5,000 per cu. mm. was slow. Clinical improvement was early and progressed favorably. In 1 patient glossitis disappeared in a week and neurologic symptoms soon regressed. A third patient who has recently been placed on the same treatment shows a similar clinical and hematologic response and an optimal reticulocytosis.

Brooklyn Veterans Hospital,
and
Columbia Memorial Hospital
New York, N. Y.

53. MEYER, L. M., KRIM, M., and SAWITSKY A.: *Oral treatment of pernicious anemia with swine duodenal mucosa and vitamin B₁₂*, Proc. Soc. Exper. Biol. & Med. 73 565-568, April 1950.

Two cases are reported confirming Bethell's finding that the hypoglossal desiccated swine duodenal mucosa

and vitamin B₁₂ is effective in the oral treatment of pernicious anemia. The daily dosage in these cases was 2 Gm of an aqueous extract of swine duodenal mucosa (given for a week before vitamin B₁₂ was begun, to determine whether this extract had any effect when administered alone) and 10 mcg. of vitamin B₁₂. In both cases, a suboptimal reticulocyte response was obtained and hematologic and clinical improvement both were satisfactory.

Columbia Memorial Hospital
Brooklyn, N. Y.

54. MEYER, L. M., SAWITSKY A., RITZ, N. D., and KRIM, M.: *Oral treatment of pernicious anemia with subminimal doses of folic acid and vitamin B₁₂*, Am. J. Clin. Path. 20 454-457 May 1950.

Although the minimal effective oral dose of vitamin B₁₂ has been found to be 75 to 150 mcg. a day the combination of 25 mcg. of vitamin B₁₂ a day and 1.67 mg. of folic acid produced good hematologic response and clinical improvement in 8 patients with pernicious anemia in relapse. Folic acid acted like "intrinsic factor" in that it promoted utilization of subminimal oral doses of B₁₂.

An addendum reports 3 additional patients who have responded well to this treatment. In one case the dosage was the same as in the first 8 patients; in the other 2, 0.67 mg. of folic acid and 10 mcg. of vitamin B₁₂ daily by mouth produced optimal reticulocytosis and rapid rise in hemoglobin and erythrocytes. Signs of clinical relapse occurred within three days after treatment was begun.

New York University College of Medicine,
New York, N. Y.

55. MEACHAM, G. C., VIGNOS, P. J., HEINLE, R. W., WEISBERGER, A. S., and EPSTEIN M.: *Vitamin B₁₂ concentrate in the maintenance of pernicious anemia*, J. Lab. & Clin. Med. 35 713-720 May 1950.

Thirty patients with Addisonian pernicious anemia were treated with vitamin B₁₂ concentrate for periods of 5 to 14 months. In the dose used (1 mcg. daily by intramuscular injection every three to four weeks) the vitamin B₁₂ concentrate did not maintain optimal blood levels. In 20 of the 30 patients erythrocyte counts under 4,000,000 were observed at the end of the treatment period. Of 26 patients previously treated with folic acid or liver extracts, 17 had erythrocyte decreases of over 500,000 per cu. during treatment with vitamin B₁₂ concentrate.

The authors consider several explanations for the failure to maintain optimal blood levels. The most probable one is that an average dose of 1 mcg. daily is not sufficient.

In no patient did neurologic or lingual relapse develop even though blood levels were not optimal. One patient who had pronounced neurologic symptoms at the start of therapy was considerably improved after treatment with the B₁₂ concentrate. No toxic or allergic symptoms due to the B₁₂ concentrate were observed.

Western Reserve University School of Medicine, and
University Hospitals
Cleveland, Ohio

56. LAMBLING, A., CONTE, M., DERRAY, J., and PÉQUIGNOT, G. Deux malades de Biermer traités par la vitamine B₁₂ (Two cases of Biermer's disease treated with vitamin B₁₂). Bull. Soc. Méd. hôp. Paris 66 722-727 May 1950.

57. BERTRAND-FONTAINE, T., MALLARME, J., and SCHNEIDER, J. Six cas de maladie de Biermer traités par la vitamine B₁₂ (Six cases of Biermer's disease treated with vitamin B₁₂). Bull. Soc. Méd. hôp. Paris 66 614-684, May 1950.

58. MORISSETTE, L. A propos de B₁₂ (Regarding B₁₂). Union méd. du Canada 79 534-536, May 1950.

The author reports satisfactory hematologic results in a case of pernicious anemia treated with vitamin B₁₂ in a neurotic syndrome with little anemia, the response was very encouraging.

The author thinks that B₁₂ should be reserved for the treatment of macrocytic anemias because it is less effective than liver as a general tonic and is therefore of less value in the treatment of hypochromic and other secondary anemias.

John-Bernard H. Campbell
Montreal, Canada

59. SEIFF, M., and REICH, C. Effect of vitamin B₁₂ administered subcutaneously. New York State J. Med. 50 1401-1402, June 1, 1950.

Almost complete remission of pernicious anemia in relapse was obtained in 2 patients who were given vitamin B₁₂ subcutaneously; the initial dosage was 25 mcg. once a week. Total dosages of 95 and 180 mcg. of vitamin B₁₂ were given in these two cases.

Response was as rapid as that obtained from intramuscular administration of vitamin B₁₂ or liver, and there was no pain or reaction from the injection. An additional report 2 other cases successfully treated with this method.

Chas. H. H. Campbell
New York, N. Y.

60. UNCLEY, C. C. Thymidine and vitamin B₁₂ in pernicious anaemia. Lancet 1: 164-165, Jan. 22, 1949.

A patient with Addisonian pernicious anemia did not respond to thymidine but responded to a small dose of the red crystalline anti-pernicious anemia factor.

61. HALL, B. E., MORGAN, E. R., and CAMPBELL, D. C. Oral administration of vitamin B₁₂ in pernicious anemia. I. Presence of intrinsic factor in Berkeley filtered pooled human gastric juice: preliminary report. Proc. Staff Meet., Mayo Clin. 24 99-107 Feb. 16, 1949.

Experiments with the oral administration of vitamin B₁₂ in 4 cases of pernicious anemia in relapse are reported. A single oral dose of 25 mcg. in one case and 5 mcg. daily in another failed to produce a hematologic response. Similarly Berkeley filtered pooled human gastric juice, from patients with uncomplicated duodenal ulcer or functional complaints referable to the gastrointestinal

tract, administered orally in a quantity of 150 cc. a day for six days, appeared to be devoid of hemopoietic activity. However a hemopoietic response was obtained in each of 3 patients who were given the filtered gastric juice simultaneously with, or within two hours of, the oral administration of 5 mcg. of the vitamin. Gastric juice obtained from the sources described above and passed through a Berkeley filter must, therefore, contain the intrinsic factor. The hemopoietic response was obtained when 150 cc. or 25 cc. of gastric juice was administered, but not when the quantity was reduced to 5 cc.

Although the potentiation of orally administered vitamin B₁₂ by an intrinsic factor in gastric juice appears to be established, the authors believe that until the vitamin has been administered orally in much larger amounts than have so far been possible, the conclusion that this vitamin has no hemopoietic activity in pernicious anemia when administered alone is not justified.

62. JONES, E., TILLMAN, C. C., and DARBY, W. J. Observations on relapses in pernicious anemia. Ann. Int. Med. 30 874-880, Feb. 1949.

Of 12 patients with pernicious anemia that had responded to liver extract therapy alone, 6 had no hematologic relapse over a period of 26 to 29 months after the treatment was discontinued. The other 6 suffered relapses only after 8 to 18 months without further treatment. Standards for hematologic relapse are described which relate relapse to the individual patient's blood level before treatment was stopped. Output of urobilinogen in the stools increases early in hematologic relapse and appears to increase further as the anemia progresses. This indicates that hemolysis becomes operative early in the relapse of pernicious anemia and emphasizes its importance in the pathogenesis of the disease.

Franklin, Penn.

63. WALDENSTROM, J. Vitamin B₁₂. Nord. med. 20: 887-890, March 30, 1949.

Author's summary: "A brief account of the development of our theoretical knowledge about liver extracts and B₁₂ is given. The clinical results obtained with the newly isolated cobalt-containing anti-pernicious anemia factor from liver (Vitamin B₁₂) are summarized. The product from Merck (Cobalene) is made more of excellent therapeutic effect in pernicious anemia, even extremely small doses are summarized. The of this substance is of importance. It seems probable that of the nervous system as it has been observed that it leads to recent nervous lesions in pernicious anemia. Its protective and general therapeutic value remains to be established. Nine cases of pernicious anemia in Uppsala, who had become sensitized against liver extracts and showed no reaction with B₁₂, with several other extracts, showed therapy of pernicious anemia will remain that the future supervision of the nervous system may be given; if neurologically symptoms develop, therapy with vitamin B₁₂ will probably be effective and harmless."

L. J. J. J. J.

64. WALDENSTRÖM J., and NORÉN B.: *Vitamin B₁₂ (den antianemiska leverfaktorn) och allergi mot leverextrakt (I vitamin B₁₂, the anti-anemia factor in liver in cases of sensitivity to liver extract)* Särtryck ur Svenska Läkartidn. No. 3, 1919

Authors summary: "The possibility that the pure antianemic factor in liver extracts may be the allergen that causes reactions after prolonged injection therapy is discussed. If such cases could be found they might be regarded as excellent test objects for the detection of the presence or absence of the antianemic factor in different preparations.

"Eight patients with pernicious anemia who had been treated for long periods with liver injections, showed positive skin reactions when tested with several commercial liver extracts. Another patient was not only sensitive in liver but also to bovine protein. All nine patients showed no cutaneous reactions with pure vitamin B₁₂ from Merck Co.

"It thus seems probable that allergic reactions are not usually caused by the liver factor and that the pure active substance may be used in future for the treatment of such allergic patients."

65. REISNER, E. H., JR.: *Treatment of pernicious anemia with crystalline vitamin B₁₂*, Am. J. Med. 6: 505, April 1949 (In Soc. Proc.)

West and Reisner treated 11 patients suffering from pernicious anemia in relapse with vitamin B₁₂. Maximal reticulocyte responses were obtained from a single injection of as little as 5 mcg.

The potency of the material as determined by these studies is slightly more than 1 International unit per microgram. Five of the 11 patients had combined system disease. The early cord lesions were completely relieved and later manifestations were greatly improved by the administration of vitamin B₁₂. Full restoration of the blood count occurred in patients maintained on this therapy. The relationship between vitamin B₁₂, folic acid, and thymidine is not yet known.

66. SCHIEVE, J. F., and RUNDLES, R. W.: *Response of lingual manifestations of pernicious anemia to pteroylglutamic acid and vitamin B₁₂*, J. Lab. & Clin. Med. 34: 439-447 April 1949

The therapeutic effectiveness of synthetic pteroylglutamic acid in pernicious anemia is reviewed with particular reference to the lingual manifestations of the disease. In the authors' cases, a few of which are reported in detail, lingual abnormalities have disappeared completely in some instances with administration of pteroylglutamic acid, and in others such treatment has had little effect.

Two patients developed severe lingual relapses while taking 30 and 50 mg. of pteroylglutamic acid daily in spite of satisfactory hematologic and neurologic responses. In these 2 cases soreness of the tongue disappeared and papillary regeneration occurred within a week following a single intramuscular injection of vitamin B₁₂, 0.025 mg. in one case and 0.050 mg. in the other. In 5 previously untreated cases of pernicious anemia with lingual manifestations a similar response was observed following administration of 0.001 mg. of the vitamin daily or 0.010 mg. in a single injection.

Duke University School of Medicine
Durham, N. C.

67. WEST R., and REISNER, E. H., JR.: *Treatment of pernicious anemia with crystalline vitamin B₁₂*, Am. J. Med. 6: 643-650 May 1949

Eleven cases of pernicious anemia, including the 3 cases previously reported (Abstr 33) have been treated parenterally with crystalline vitamin B₁₂. All have shown hematologic improvement, with blood counts rising to normal levels when weekly doses of 25 mcg. or less were given. The minimum effective dose has been found to be approximately 1 mcg. a day intramuscularly. Five of these patients had combined system disease; none of them became worse, as has sometimes occurred during folic acid treatment, and all are now able to walk. Improvement varied in degree in the different cases. Reports of the 11 cases are given. The current status of knowledge regarding the relationship of thymine, folic acid, cobalt, and vitamin B₁₂ is discussed. Cobalt salts were given to one of the patients in this series without effect. Vitamin B₁₂ appears to be the erythrocyte maturation factor of liver.

Columbia University and
Bellevue Hospital
New York, N. Y.

68. WEST R.: *The treatment of Addisonian pernicious anemia with vitamin B₁₂*, A.M.A. Scientific Assembly Official Program, June 1949 p. 95. (See also Abstr 67)

"Eleven patients with Addisonian pernicious anemia treated parenterally with vitamin B₁₂ all showed a return of all the elements of the blood count to normal or slightly higher than normal levels. Five patients with spinal cord lesions of less than six months duration showed improvement of the cord lesions, including a return to normal of the Babinski reflex in the 1 case in which this reflex was abnormal. The minimal dose producing a maximal reticulocyte and blood response was 1 microgram a day by injection, smaller doses giving less than maximal responses. The effective oral dose appears to be considerably larger than the parenteral dose. Several cases of aplastic anemia, both idiopathic and secondary to drugs, have shown no response."

New York, N. Y.

69. JIMÉNEZ DE ASOJA, F.: *La vitamina B₁₂ en el tratamiento de la anemia perniciosa (Vitamin B₁₂ in the treatment of pernicious anemia)* Rev. Soc. argent. de hemat. y hematop. 1: 65-77 June 1949

The author presents a review of vitamin B₁₂ together with reports of 4 cases of pernicious anemia treated with this agent. The results agreed with those obtained elsewhere. Great activity was shown by small quantities of vitamin B₁₂; in one patient who received a total of 50 mcg. in a little over a month, the erythrocyte curve was comparable to that produced in 4 other patients by 25 mcg. of folic acid a day by mouth. The author believes that if larger doses were used—perhaps tenths of milligrams instead of micrograms—more lasting results would be obtained. Future treatment may consist of vitamin B₁₂ with pteroylglutamic acid.

Hematologic Service of Casero College
Buenos Aires, Argentina

70. PINEY A., and BARKLEY T. Y.: *Sensitivity to liver* Lancet 1 1119 June 25, 1949 (in Letters to the Editor)

A patient with pernicious anemia became hypersensitive to liver extract after receiving it parenterally for about four years. Protolyzed liver by mouth was substituted, but this did not have so satisfactory a hematologic effect, and it became extremely distasteful to the patient after several months. Anthraquin (Neo-Antergan) did not prevent the allergic reaction to parenteral liver when an attempt was made to resume such treatment. An injection of 20 mcg. vitamin B₁₂ and three further injections of this material (amount not stated) caused no untoward reactions. The patient's general condition improved and slight neurologic abnormalities almost disappeared.

71. WALDENSTRÖM, J., and NÖREN, B.: *Vitamin B₁₂ den antiänemiska leverfaktorn och allergi mot leverextrakt (Allergy to liver extracts and vitamin B₁₂, the antianemic factor of liver)* Svenska läk. sällsk. förhändl. 46 157 160, Fasc. 3 1949 (abstr. Quart. Rev. Allergy 3 87 June-Sept. 1949)

Nine patients who were allergic to commercial liver extracts were tested with vitamin B₁₂ intracutaneously. Nine had a positive reaction. The test dose of vitamin B₁₂ was about 1/100 of a full therapeutic dose (0.015 mg.) but much greater than the test doses of the commercial liver extracts. These results seem to confirm the view that allergy to liver extract is not attributable to the antianemic factor itself.

*Department of Tropical
Diseases, Sweden*

72. KAUFMANN J., and COOPERBERG, A.: *Vitamin B₁₂ in pernicious anemia*, Canad. M. A. J. 60 552-554, June 1949

Two patients with pernicious anemia were successfully treated with small doses of vitamin B₁₂ given at repeated intervals. The dose given at each injection is not specifically mentioned, although the initial dose in one patient was 15 mcg., and in the other 25 mcg. The average daily dose thereafter was scarcely over 1 mcg. The reticulocyte and hematologic response of these patients to vitamin B₁₂ was as adequate as could be obtained with the most potent liver extract.

The first patient had considerable central nervous system involvement, and the neurologic symptoms appeared as became aggravated after vitamin B₁₂ was started and during the period of hematologic improvement, but disappeared with continued treatment.

73. CHALMERS, J. N. M.: *Sensitivity to liver* Lancet 2 83, July 9 1949 (in Letters to the Editor)

The writer used vitamin B₁₂ derived from *Streptomyces griseus* (Glaxo Laboratories) in 2 patients with pernicious anemia who experienced severe reactions to liver extract given intramuscularly. Both patients, who have objective manifestations of subacute combined degeneration of the cord, showed a good hematologic response, and their neurologic symptoms also seem to have improved. Repeated intramuscular injections of 12 mcg.

of the B₁₂ preparation have caused no constitutional disturbances or allergic reactions.

*St. George's Hospital
London, England*

74. REISNER, E. H., JR.: *The present status of vitamin B₁₂ in pernicious anemia*, Bull. New York Acad. Med. 25 429-433 July 1949

This paper is largely a review of vitamin B₁₂ clinical studies. The experience with patients in the First and Fourth Medical Divisions of Bellevue Hospital is summarized as follows: "It was found that a single injection of as little as 3 to 6 micrograms produced a maximal reticulocyte response, and a total dose of 56 micrograms in one patient effected complete hematologic remission, which was maintained for four months before relapse, with no additional therapy. A dose of one microgram a day was found to give a maximal reticulocyte response and restore the blood count to normal. A daily injection of 1/10 of a microgram was ineffective, but remission occurred when this was increased to 3/4 of a microgram daily. One-half a microgram daily gave a submaximal reticulocyte response. From three experiments the unit potency of B₁₂ was fixed at approximately one unit per microgram, a unit being defined as the amount of antianemic substance required daily to effect and maintain hematologic remission in a patient with pernicious anemia. One of the Bellevue patients had exhibited early cord lesions at the onset of treatment with one gamma a day and at the end of fifty-three days of treatment had a negative neurological examination."

*Bellevue Hospital
New York, N. Y.*

75. MEYER, L. M., RITZ, N. D., ROWEN M., BOCK, G., and RUTZKY J.: *Treatments of pernicious anemia with animal protein factor concentrates of bacterial origin*, Bull. New York Acad. Med. 25 464, July 1949 (in Soc. Proc.)

A.P.F. 60 and Normocytin, two animal protein factor concentrates prepared from the aerobic fermentation of a bacillus isolated from chicken feces, were tested for antipernicious anemia effect in patients with pernicious anemia in relapse. By chick assay A.P.F. 60 had biologic activity equal to "10 unit liver" per cc. and Normocytin a potency of 10 mcg. of vitamin B₁₂ per cc. By *Lactobacillus leichmannii* assay their respective potencies were 65 per cent of "10 unit liver" and 8 mcg. of vitamin B₁₂ per cc. A.P.F. 60 induced a satisfactory reticulocyte response in 4 of 5 patients and an increase of hemoglobin and red blood cells in all 5. Normocytin had these three effects in all 8 patients, normal hematologic values being reached in 6, including one patient who previously had received A.P.F. 60. All the patients experienced a feeling of general well-being, megaloblastic marrows were converted to normal, neurologic changes when present were improved, and there was no instance of progression or development of nervous system signs or symptoms.

76. BORTZ, D. W.: *The value of vitamin B₁₂ in pernicious anemia preliminary report*, Cleveland Clin. Quart. 16 148-153, July 1949

The first 5 cases of pernicious anemia treated with vitamin B₁₂ at the Cleveland Clinic are reported. All the

patients experienced a sense of well-being soon after this treatment was begun—one of them within 12 hours. The dosage varied widely 10 or 25 mcg. being injected at intervals of one day to one month. At the time of this report an attempt was being made to maintain the patients on a dose of 10 mcg. at two-week and four week intervals. Under vitamin B₁₂ therapy the red blood count and hemoglobin rapidly return to normal, but there is a lag in the fall of the volume index to normal levels. Leukopenia, when present, clears up slowly.

An addendum states that 3 additional patients with pernicious anemia have been placed on vitamin B₁₂ therapy. One with severe pyramidal tract involvement has responded well to intensive treatment. In the second, normal hematologic values have not been obtained, and coexistent liver disease is suspected. The third patient, who has carcinoma of the prostate, is responding satisfactorily.

Clareland, Glass
Clareland, Glass

- 77 ERF L. A., and WIMER, B. Comparison of vitamin B₁₂ from liver and from *Streptomyces griseus* in the treatment of pernicious anemia, *Blood* 4: 845-862, July 1949

A patient with pernicious anemia in relapse responded hematologically and clinically to two injections on successive days of 25 mcg. each of vitamin B₁₂ from liver. The case was followed for 114 days. The erythrocyte peak (3,900,000 cells/cu. mm.) occurred on the 86th day. Two other patients with pernicious anemia in relapse who were treated with vitamin B₁₂ derived from *Streptomyces griseus* experienced hematologic and clinical responses similar to those obtained by other investigators with vitamin B₁₂ from liver in dosages of the same magnitude. One of these two patients had no neurologic complaints and received a single injection of 32 mcg. the other had severe subacute combined degeneration and was given two doses of 32 mcg. each, nine days apart. Definite neurologic improvements were seen in the latter case. Results in these 3 cases indicate that the factor from liver and that from *Streptomyces griseus* are closely related or identical.

Four of 5 patients with pernicious anemia in remission who had been receiving liver extract regularly reported subjective improvement following a single intramuscular dose of 32 mcg. of vitamin B₁₂ from *Streptomyces griseus*, and 1 of the 4 showed a significant rise in the erythrocyte count.

The authors, in an addendum to their article, present further observations on these 8 patients and data on 3 additional cases of pernicious anemia in which vitamin B₁₂ from *Streptomyces griseus* was administered. Some of the initial 8 patients were subsequently given additional vitamin B₁₂ by injection or sublingually others were placed on liver therapy, and some required no further treatment at the time of writing. Allowing for individual variations, it is estimated that 50 to 100 mcg. of the vitamin given by injection will produce a remission in pernicious anemia lasting for 50 to 100 days, and that about 50 mcg. given during the remission will maintain it for 70 to 120 days. General and neurologic complaints appear more difficult to control than the anemia. However

glossitis consistently responds to treatment. That vitamin B₁₂ therapy occasionally results in iron deficiency as remission occurs was exemplified in one case the deficiency was overcome by intravenously administered iron.

The results of sublingual therapy were inconclusive since the patients were in partial remission when this treatment was given. Slight reticulocytosis occurred in 2 of 3 trials of sublingual therapy (dosage 50 or 200 mcg.) and there was definite clinical improvement in each case.

78. BETHELL, F. H., SWENDSEID, M. E., MEYERS, M. C., NELIGH, R. B., and RICHARDS, H. G. Observations on the hemopoietic factors in hog stomach and duodenum, and the treatment of pernicious anemia by orally administered vitamin B₁₂ in combination with extracts of duodenal mucosa, *Univ. Hosp. Bull., Ann Arbor* 13: 49-53, July 1949

A review of the literature is presented, showing that vitamin B₁₂ in dosages sufficient to produce an optimal therapeutic response when administered parenterally to patients with pernicious anemia in relapse, is essentially ineffective when given by mouth. Orally administered vitamin B₁₂, however, has proved effective when given in combination with human gastric juice, which indicates that vitamin B₁₂ and Castle's extrinsic factor are either identical or closely related substances.

Since desiccated hog stomach and duodenum given orally are effective in pernicious anemia, it is considered that their activity might be due to a combination of vitamin B₁₂ and intrinsic factor. Microbiologic assay demonstrated in desiccated hog stomach a considerable amount of vitamin B₁₂ which was not destroyed by heat. The vitamin B₁₂ content was also confirmed by clinical response in 2 patients with previously untreated pernicious anemia.

Further clinical trials were performed using desiccated extracts of hog duodenal mucosa. One patient was first treated ineffectively with 5 mcg. of crystalline vitamin B₁₂ a day given orally in two divided doses, and one with 10 Gm. a day of nondialyzable lyophilized duodenal mucosa extract containing both the soluble and insoluble fractions. In both cases a satisfactory hemopoietic response was obtained from the combined use of the two substances. A third patient failed to respond to 5 mcg. of vitamin B₁₂ in the form of a concentrate given orally for six days, but responded to this treatment in combination with 1.5 Gm. of the lyophilized soluble portion of the nondialyzable duodenal mucosa extract.

These findings provide evidence that vitamin B₁₂, or a closely related substance, and intrinsic factor constitute the effective hemopoietic agents in hog gastric and duodenal tissue.

Ann. Arbor Mich.

- 79 BEARD, M. F., NATARO, M., and LAYMAN, L. H. Vitamin B₁₂ in pernicious anemia, *South. M. J.* 42: 677-684, Aug. 1949

Six cases of pernicious anemia in relapse were treated with vitamin B₁₂ intramuscularly with a view to determining the minimum dosage that will give an

mum hematologic response and observing the effect of this vitamin on the neurologic manifestations of pernicious anemia in relapse. All 15 patients showed hematologic improvement. The reticulocyte and erythrocyte responses were comparable to those observed in patients treated with liver and 1 microgram of vitamin B₁₂ appeared to be approximately equivalent to 1 U.S.P. unit of refined liver extract. To correct depletion of hemopoietic substance in the liver and bring about normal storage, an initial injection of 0.2 to 0.3 mg. of vitamin B₁₂ followed by monthly injections of 0.03 mg. is suggested. In the authors' cases, change from a megaloblastic to a normoblastic type of bone marrow was almost complete 48 to 72 hours after the administration of 0.025 or 0.05 mg. of vitamin B₁₂, and the change began six hours after the administration of 0.1 mg. in one patient. In 5 cases atrophy of lingual mucosa disappeared within four or five weeks and glossitis disappeared within 10 days; the sixth patient did not have these manifestations. Minimum peripheral neuritis, present in 5 cases, improved moderately within four to six weeks. The combined sclerosis in 2 cases showed subjective improvement, but little change in the objective neurologic signs.

80. REISNER, E. H., JR., and WEST, R. *Effect of thyminase deoxyriboside (thyminase) on human pernicious anemia*. Proc. Soc. Exper. Biol. & Med. 71: 651-652, Aug. 1949

Two patients with Addisonian pernicious anemia in relapse were given single injections of 5.3 mg. and 150 mg. of thyminase, respectively. The reticulocyte count rose, but not the red cell count. One week later daily intramuscular injections of 1 mcg. of vitamin B₁₂ were begun, which produced a further rise in reticulocytes and an increase in the erythrocyte count. A third patient was given a suboptimal intramuscular dose of 0.25 mcg. of vitamin B₁₂ daily. The number of reticulocytes increased and then declined and the number of red blood cells increased. When 5 mg. of thyminase was given daily for nine days in addition to vitamin B₁₂ there was a second rise in reticulocytes and red blood cells, and when thyminase was dropped the red blood cell count declined. It appears that thyminase is not completely inert hemopoietically but if it has an enhancing effect on B₁₂ activity this is very slight. In view of the ability of other deoxyribosides to substitute for vitamin B₁₂ in bacterial growth, the effect of mixtures of deoxyribosides on pernicious anemia should be investigated.

81. MEYER, L. M., RITZ, N. D., CACCISE, A., RUTKY, J., SAWITSKY, A., and BOCK, G. *Studies in pernicious anemia patients treated with liver extract and folic acid antagonists*. Am. J. M. Sc. 218: 197-203, Aug. 1949

Five patients with pernicious anemia showed no hematologic responses when folic acid antagonists (pteroylseric acid and methyl pterotic acid) and adequate doses of liver extract were administered simultaneously. This suggests that pteroylglutamic acid is necessary for the production of red blood cells. No clinical remission or reticulocyte response was obtained in a patient with pernicious anemia in relapse following intramuscular administration of 10 and 15 mcg. of vitamin B₁₂ with 5 mg. of α -methopterin [sic] (4-amino-10-methyl pteroylglu-

tamic acid) on the third and eleventh days of a 14-day course of treatment with a folic acid antagonist (methyl pterotic acid); the hemoglobin level and red and white blood cell counts did not rise, and sternal aspirations on four occasions revealed a megaloblastic of 11 to 23 per cent.

82. SCHIEVE, J. F., and RUNDLES, R. W. *Response of lingual manifestations of pernicious anemia to pteroylglutamic acid and vitamin B₁₂*. Am. J. Med. 7: 256-257 Aug. 1949 (in Soc. Proc.)

Pteroylglutamic acid may fail to induce and maintain remissions of the lingual manifestations of pernicious anemia, as well as of the anemic and neurologic manifestations. Of 7 patients with pernicious anemia in relapse who had lingual mucosal atrophy, only 2 responded with regeneration of the filiform papillae in seven to ten days of treatment with 80 to 100 mcg. of folic acid a day. In the remaining 5, the lingual response was poor and 2 of them had definite lingual relapses during the third month of therapy. When these 2 in relapse were given intramuscular injections of 0.010 and 0.025 mg. of vitamin B₁₂, the filiform papillae regenerated and the color of the tongue became normal in six and seven days, respectively. In 5 patients with untreated pernicious anemia in relapse who also had lingual manifestations of the disease, regeneration of the papillae and restoration of a normal lingual color occurred six to ten days after treatment with a single dose of 0.001 mg. of vitamin B₁₂ or with 0.0001 mg. daily.

Duke University School of Medicine
Durham, N. C.

83. JACOBSON, B. M. *The treatment of pernicious anemia*. M. Clin. North America 29: 1385-1400, Sept. 1949

This article contains a section in which the therapeutic efficacy of vitamin B₁₂ is evaluated. A tabulation is presented of the effect of this vitamin on erythrocyte regeneration as reported in the literature by several investigators and as found in the author's own experience (4 cases). The author concludes that 1 mcg. of vitamin B₁₂ is not equivalent to 1 U.S.P. unit of liver extract, and that 2 mcg. a day does not result in a maximal hemopoietic effect not equal the effect produced by 1.5 U.S.P. units of liver extract administered daily.

The use of commercial preparations of vitamin B complex containing small amounts of folic acid in the treatment of pernicious anemia is deprecated, for in early cases a small amount of folic acid may mask the early symptoms and early hematologic changes and render diagnosis difficult.

Harvard Medical School
Boston, Mass.

84. MARTIN, E., and VAUCHER, F. *Remarkable activity of proteolyzed liver extract in pernicious anemia*. Praxis 38: 802-803, Sept. 15, 1949 (abstr. J.A.M.A. 142: 446, Feb. 11, 1950)

The authors report on a liver extract which has undergone proteolysis under the influence of papain. This enzymatic action digests the proteins up to the polypeptide stage and makes it possible for the patient with pernicious

anemia to utilize Castle's intrinsic factor. There is a solid and a liquid proteolyzed liver extract for oral use, as well as a highly concentrated proteolyzed extract for parenteral use. The latter was used in 15 patients with hyperchromic, macrocytic anemia. In patients with pernicious anemia, 2 cc. of the extract are injected per week. In the preparation of this extract some of the vitamin B factors are lost, but the vitamin B₁₂ content remains high. This proteolyzed extract is two to three times more potent than ordinary whole liver extracts.

85. KOHARI KUCHARIK, J. *Reticulocyte response to liver therapy in pernicious anemia*, Lancet 2 1034-Dec. 3, 1949

86. UNGLEY C. C. *Vitamin B₁₂ in pernicious anaemia: parenteral administration*, Brit. M. J. 2 1370-1377 Dec. 17 1949

Responses to 73 single doses of vitamin B₁₂ injected intramuscularly were studied in 53 patients with pernicious anemia in relapse. Care was taken to ensure that the responses were not due to dietary factors or spontaneous remissions, and a test was devised to measure the accuracy of the blood counts. The doses of vitamin B₁₂ were graded logarithmically from 1.25 to 160 mcg. The reticulocyte response was compared, in 39 patients, with the expected response to liver extract (Issacs and Friedman, *Am. J. M. Sc.* 196 718, 1938) but a better criterion of the response was the increase of red blood cells in 15 days.

By both measures of evaluation noted, a single dose of 10 mcg. of vitamin B₁₂ produced as satisfactory a response as is usually obtained with liver extract. Larger doses produced an even greater increase of red blood cells in 15 days. The amount required to raise the red blood cell count above 4,500,000 per cu. mm. in 82 cases varied from 15 to 140 mcg. These amounts also led to an increase in white cells and platelets and to clinical recovery. A maintenance dosage of 10 mcg. every two weeks was satisfactory in all but 8 of 21 patients followed for 6 to 15 months. The 3 exceptions were patients in whom soreness of the tongue was not relieved (one also had macrocytosis). Six women developed iron deficiency after 8 to 15 weeks, which was relieved by ferrous sulfate. No new neurologic symptoms appeared, and those already present improved or remained unchanged. The results of vitamin B₁₂ therapy in 8 patients with subacute combined degeneration—usually 40 mcg. weekly for at least 10 months—were as satisfactory as those obtained in a previous series of cases with crude or refined liver extract.

Ungley suggests a dosage schedule of 40 to 80 mcg. of vitamin B₁₂ initially followed by 20 mcg. weekly for three months and 8 mcg. every three weeks thereafter for uncomplicated cases of pernicious anemia, and at least 40 mcg. weekly for six months followed by 20 mcg. weekly thereafter for patients with subacute combined degeneration. Some patients will need more, and at the least sign of relapse—hematologic, lingual, or neurologic—or if infection develops, the dosage should be temporarily doubled or trebled.

Read Victoria General Hospital, Newcastle-upon-Tyne, England

87. MILLS J., and HEMSTED E. H. *Treatment of pernicious anemia*, Lancet 2: 1151 Dec. 17 1949 (In Letters to the Editor)

All of 14 patients in their first attack of pernicious anemia showed a vigorous reticulocyte response to vitamin B₁₂, which was followed by a rapid return of hemoglobin and red cells to normal. Eighteen patients who had relapsed in spite of liver treatment were also successfully treated with the vitamin. A few patients under liver maintenance but not in relapse volunteered that they felt better when they changed to vitamin B₁₂ therapy and generally their red cell levels rose from 4.0 to 4.5 million, to 5.0 million per cu. mm. Such consistent results have not been obtained with any of the liver extracts at present available in England.

Reading, England

88. PAPPWORTH, M. H. *Vitamin B₁₂ (from Streptomyces griseus) in pernicious anaemia*, Brit. M. J. 1 1302-1303, June 3, 1950 (In Medical Memoranda)

Each of 2 patients with pernicious anemia was given an injection with 3 cc. of a concentrate from the metabolite fluid of a strain of *Streptomyces griseus*. An excellent response was obtained in both patients.

89. MEYER, L. M., RITZ, N. D., ROWEN, M., BOCK, G., and RUTZKY J. *Treatment of pernicious anemia with animal protein concentrates of bacterial origin*, Acta haemat. 3 305-322, June 1950.

90. HICKS, D. G. T. *The treatment of pernicious anemia*, Post-Grad. M. J. 29 305-307 June 1950.

91. NOREN B. *Allergic reactions in parenteral liver therapy and vitamin B₁₂*, Acta med. Scandinav. 137 48-65 Feb. 7 1950 (abstr. Quart. Rev. Allergy 4 80 June 1950)

Twenty four (18 per cent) of a series of 130 patients with pernicious anemia, treated with parenteral liver extract, manifested allergic reactions, the most common of which were itching and nausea. Only 5 patients had respiratory symptoms. On being tested with different commercial liver preparations, 14 more of the 130 patients responded with positive skin reactions. Since they had no clinical symptoms, they have been classified by the author as "latent allergics." All of the 24 patients with clinical allergic symptoms to liver extract were able to tolerate vitamin B₁₂ without any allergic reactions.

In an editorial note it is stated that this article is significant in that a large number of patients were carefully observed and vitamin B₁₂ is proved to be nonallergenic. The classification of "latent allergics" is criticized, it being stated that the existence of latent allergy is problematic. Reference is made to the existence of a great number of persons who do not become sensitized despite repeated parenteral injections of liver extract known to sensitize others.

University Hospital
Uppsala, Sweden

92. YOUNG W. C., ULRICH, C. W., and FOUTS P. J. *Sensitivity to vitamin B₁₂ concentrate*, J.A.M.A. 143 893-894, July 8, 1950.

A patient with pernicious anemia was highly sensitive to liver extracts from both beef and pork liver. A vitamin B₁₂ concentrate made from streptomycin broth was given in doses equivalent to 5 mcg. of B₁₂ every five days. After the sixth dose the patient complained of burning of the throat and itching and swelling at the injection site. Five or six more injections were given and similar discomfort was experienced each time. Medication was then changed to a vitamin B₁₂ concentrate made from liver and 5 mcg. was given every five days for six months without untoward reaction. Because the supply of this concentrate was exhausted, a vitamin B₁₂ concentrate made from streptomycin broth was again given. About 5 to 10 minutes after the patient received the eleventh injection, peripheral circulatory collapse developed. Sodium chloride infusions, epinephrine injections and oral administration of antihistaminic drugs brought about recovery in about three hours. Therapy was then changed to crystalline vitamin B₁₂ made from streptomycin broth, and the patient has shown no evidence of sensitivity to this form of the vitamin.

Journal of General Internal Medicine
Philadelphia, Pa.

93. SCOTT R. B.: *Diagnosis and treatment of pernicious anemia*, Brit. M. J. 2 157-159 July 15, 1950.

A retroader course for general practitioners.

94. SCHURMPP A.: *B₁₂ and folic acid in small doses in pernicious anemia*, Nord. med. 44 1197 July 22, 1950 (abstr. J.A.M.A. 144 1039 Nov 18, 1950)

Six patients with pernicious anemia were treated with a combination of vitamin B₁₂ and folic acid given orally in minimal doses. In 3 cases the daily dose was 10 mcg. of vitamin B₁₂ and 0.67 mg. of folic acid in 2 cases the amount was doubled after 5 and 11 days respectively and in 1 patient the small dose was ineffective and liver extract was given parenterally followed by vitamin B₁₂ and folic acid in increased doses. With successful vitamin B₁₂ and folic acid therapy about 24 per cent of the hemoglobin deficit was covered, and a hemoglobin rise of 13.4 per cent every 10 days was attained. The reticulocyte reaction was later and less apparent than with parenteral therapy. In all but one of the patients with leukopenia and thrombopenia, normal increase in leukocytes and thrombocytes occurred. Neurologic symptoms seemed to be relieved.

95. BORTZ, D. W., and BATTLE, J. D., Jr.: *Massive use of vitamin B₁₂ in treatment of pernicious anemia*, Cleveland Clin. Quart. 17: 166-168, July 1950.

96. STRAUSS, M. B.: *Vitamin B₁₂ and pernicious anemia*, New England J. Med. 243 187-194; 222-229 Aug. 3, and Aug. 10, 1950.

In this article are discussed and described the clinical aspects and pathogenesis of pernicious anemia, the value of vitamin B₁₂ and folic acid in treating pernicious anemia, the possible relationship of vitamin B₁₂ and the animal protein factor the relationship between vitamin B₁₂ and growth factors for rats and other animals and the role of vitamin B₁₂ in the synthesis of nucleic acid, and microbiologic assay methods for vitamin B₁₂. References on the effects of vitamin B₁₂ in pernicious anemia and on other related macrocytic anemias are reviewed. Other physiologic effects of vitamin B₁₂ in man are described, such as

its effect on growth in children, and its diuretic effect. The relation of vitamin B₁₂ to the extrinsic factor of Castle is mentioned. The importance of the differential diagnosis of pernicious anemia from other blood disorders is emphasized.

The treatment of pernicious anemia is outlined briefly. During the first week of therapy 50 U.S.P. units of liver extract or 50 mcg. of vitamin B₁₂ is given. Thereafter 15 mcg. of vitamin B₁₂ is given by injection once weekly until the blood values are normal. If there is considerable involvement of the nervous system, it is advisable to continue these weekly injections for the first year. If the nervous system is not particularly involved, the weekly injections are followed by the administration of vitamin B₁₂ every two weeks for the first year and then the frequency of injections is decreased to one in four weeks. The advantages of giving vitamin B₁₂ by injection, rather than by mouth are mentioned.

Cooking P. A. Hospital
Franklin, Mass.

97. FULD H.: *Folic acid and pernicious anemia*, Brit. M. J. 2 527 Aug. 25, 1950 (in Correspondence)

The writer agrees with Dr. Howard (Brit. M. J. 2: 417 Aug. 12, 1950) that folic acid per se is not the causal factor of neuropathy in cases of pernicious anemia treated with this agent. He does not, however, agree that folic acid should be given in combination with liver extract or vitamin B₁₂ in the treatment of pernicious anemia.

In addition, he reports a case of idiopathic stenospor rheo in which vitamin B₁₂ was highly effective after folic acid had proved ineffective.

Liverpool, England

98. BEARD M. F., McILVANE, S. K., and NATARO, M.: *Vitamin B₁₂ in pernicious anemia in remission*, South. M. J. 43: 678-683, Aug. 1950.

Treatment of 18 patients with pernicious anemia who had been maintained on refined liver therapy (120 U.S.P. units a month) was changed to 30 mcg. of vitamin B₁₂ given once a month. Six of these patients had not responded completely to liver therapy but improved under B₁₂ therapy. 5 patients improved slightly or remained in the same condition after the shift in therapy; 7 (with one exception) who had been stabilized by liver therapy showed declines in hemoglobin levels, with or without subjective complaints, when they were maintained on vitamin B₁₂ therapy. One of these patients required 100 mcg. of vitamin B₁₂ every two weeks before improvement became evident.

The results in these patients indicate that there is no definite correlation between the amount of liver extract and the amount of vitamin B₁₂ required by the individual patient. In 11 of the 18 patients a dose of 30 mcg. once a month was adequate but in the other 7 it was not. While vitamin B₁₂ is a dramatic therapeutic agent for pernicious anemia in relapse, apparently it is not a complete replacement for liver extract as maintenance therapy in some patients.

In the discussion, Dr. T. D. Spies stated that the B₁₂ concentrates used by Dr. Beard came from Streptomyces

and not from liver. While the B_{12} itself is the same in both concentrates, other substances may be present in the concentrate from liver which are effective in blood regeneration. The variabilities in source material, in methods of assay, and in the patients' make variability in results inevitable. Dr. Spies thinks of raw liver as containing folic acid (to which some patients respond who do not respond to liver extract or B_{12}), vitamin B_{12} , and other valuable nutrients. Oral vitamin B_{12} therapy is effective in some patients but not in all. The patient with pernicious anemia has enough vitamin B_{12} in the alimentary tract to be effective, but some help from liver or stomach preparations or gastric juices is required to get it through the gut wall.

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Louisville 5*

- 99 VILTER, R. W., HERRIGAN, D., MUELLER, J. F., JARROLD, T., VILTER, C. F., HAWKINS, V., and SEAMAN, A. *Studies on the relationships of vitamin B_{12} , folic acid, thymine, uracil, and methyl group donors in persons with pernicious anemia and related megaloblastic anemias*. Blood 5: 693-717 Aug. 1950.

Authors' summary and conclusions: "Patients with pernicious anemia who are maintained on folic acid, 30 mg. three times a week, for two to three years may have a hematologic relapse which will remit satisfactorily if refined liver extract is given, or partially if the dose of folic acid is increased to 50 mg. daily or if thymine is given.

"The hematologic remission succeeding the increased dosage of folic acid is followed within several months by a second relapse. At this time the response of these patients to liver extract or vitamin B_{12} is retarded. Recovery occurs after two to four months.

"These experiments suggest that folic acid exerts its effect by mass action in pernicious anemia and that it is essential in the formation of thymine and other pyrimidines and purines or facilitates the utilization of these substances.

"Porterolateral column disease or peripheral neuritis occurred in every person with pernicious anemia who received increasing doses of folic acid to maintain a hematologic remission. This observation suggests that folic acid, by pushing a chemical reaction through to completion in the face of a serious deficiency of vitamin B_{12} , depleted the supply of this factor even more and led to the development of combined system disease.

"Uracil produced a hematologic response in 2 of 3 persons with pernicious anemia in relapse when given in doses of 15-30 grams daily. The data suggest that uracil may be a precursor of thymine.

"A patient with pernicious anemia of pregnancy failed to respond to uracil, 30 grams daily but did respond partially when choline, 3 grams, and methionine, 6 grams were given. Thymine induced a complete response. The partial response to methionine and choline and the better response to thymine suggest that choline and methionine supplied methyl groups for the formation of thymine, but that activating substances for the methylating process were missing.

"Reference is made to a patient previously reported from this laboratory who had liver extract and vitamin

B_{12} -refractory megaloblastic anemia but who responded to folic acid and on a second relapse to thymine. Studies on the output of folic acid in the urine of this patient did not support the possibility of folic acid deficiency and the suggestion was made that another substance, possibly the "Wills factor" was deficient, and that this factor together with folic acid activated the formation of thymine. These two factors correspond to the activators of the transmethylation reaction mentioned in the preceding paragraph.

"These studies on human beings and similar ones in bacterial metabolism suggest that folic acid, liver extract and vitamin B_{12} are essential to the formation of nucleic acid and nucleoprotein through a chemical chain reaction. The suggestion is made that the megaloblast common to pernicious anemia and related macrocytic anemias is a primitive erythroblast with an abnormality in the metabolism of nucleoprotein. The so-called maturation arrest in all marrow elements occurs because of this abnormality which may be induced by a deficiency of vitamin B_{12} , folic acid, the "Wills factor" and probably other chemical activators of this reaction."

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Cincinnati, Ohio*

100. WILKINSON, J. F. *Vitamin B_{12}* , Lancet 2 407 Sept. 23, 1950 (In Soc. Proc.)

"Dr. J. F. Wilkinson remarked that the dosage of B_{12} is steadily on the upgrade and he now gives 20-60 μ g. weekly but, even so, glossitis and poor counts persist in some cases. Allowance must be made for big variation in individual requirement of active material in patients with pernicious anemia, hence only reasonably large series of cases are useful for estimating dosage. Dr. C. C. Ungley said he now gives a minimum of 60 μ g. weekly increasing up to 200 μ g. if there are neurological complications."

101. BEARD, M. *Vitamin B_{12}* , Lancet 2 407 Sept. 23, 1950 (In Soc. Proc.)

"Dr. M. Beard (U.S.A.) had a group of thirty-seven patients who were being treated with liver extract, not all satisfactorily. They were transferred to vitamin B_{12} treatment, 30 μ g. monthly. At first they did well, the patients with low counts rising to normal levels. But after 7-11 months the counts fell and the M.C.V. rose again. Increase of B_{12} up to 100 μ g. weekly had no effect. Equally ineffective were supplements of folic acid, pyridoxine, iron, and ascorbic acid. Most of the patients had inadequate protein in their diet, and Dr. Beard thought that a factor in liver protein might be necessary for full recovery in pernicious anaemia."

102. STRAUSS, M. B. *Modern treatment of the anemias*, M. Clin. North America 34: 1291-1304, Sept. 1950 (Boston Edition)

Vitamin B_{12} and folic acid treatment for pernicious anemia is included in this review. The dosage schedule for vitamin B_{12} is given. Initial administration is 50 mcg., then 15 mcg. is injected once weekly until the blood values return to normal. The injection is then given every two weeks for the remainder of the first year and thereafter once every four weeks. Advantages of injected vitamin B_{12} over oral administration are considered to be that the quantity required by injection is less, intestinal perme-

ability does not become a factor and treatment is kept under the control of the physician. Folic acid is not advised in pernicious anemia treatment since neurologic relapse often results, and may be made worse by increasing the dosage of folic acid. An impurity in the preparation may be responsible for these effects.

Cooking, F. A. Hospital
Farnborough, Kent.

103. UNGLEY C. C. *Vitamin B₁₂ in pernicious anemia*, *Lancet* 2: 468, Oct. 7 1950 (in Letters to the Editor)

In the report (*Lancet*, Sept. 23 [Abstr. 100]) of the International Congress of Hematology Dr Ungley was quoted as saying that he now gives "a minimum of 60 µg. (of vitamin B₁₂) weekly increasing up to 200 µg. if there are neurological complications." In this letter he describes his current dosage practices. For routine purposes he uses the cheaper vitamin B₁₂ concentrates, generally giving 60 mcg. every three weeks, with more frequent and perhaps larger doses (rarely more than 100 mcg. a week) for patients with subacute combined degeneration of the cord. He has 40 or 50 patients with little or no neurologic involvement who are receiving only 10 mcg. of vitamin B₁₂ or B₁₂ every two weeks in a study comparing the effects of these two vitamins.

Royal Victoria Infirmary
Farnborough-Surrey, England

104. UNGLEY C. C.: *Absorption of vitamin B₁₂ in pernicious anemia. I Oral administration without a source of intrinsic factor* *Brit. M. J.* 2: 905-908, Oct. 21, 1950.

Author's summary "The absorption of vitamin B₁₂ was studied by comparing the effective oral dose with the parenteral dose expected to produce a similar increase of red blood cells in 15 days. The apparent oral-dose/parenteral-dose ratio was corrected if the observed response to injected material was below expectation, indicating a relative resistance to vitamin B₁₂.

"In one case daily doses of 5 µg. gave no response. In another even 80 µg. a day for 24 days produced an increase in red blood cells no greater than would have been expected in 15 days from the injection of 2.5 µg. The response to injected material was normal, so that the corrected oral-dose/parenteral-dose ratio was several hundreds to one. By contrast the oral-dose/parenteral-dose ratio in five cases given a single dose of 5,000 µg. was from 20 to 40, the increase of red blood cells in 15 days being so great as to suggest the absorption of 80 to 160 µg. or even more. Presumably very little of the vitamin absorbed can have interacted with Castle's intrinsic factor considering the low quality and quantity of the secretion of the stomach in pernicious anemia.

"Further tests are necessary to determine whether in fact more vitamin B₁₂ is absorbed after a single large dose than after the same quantity of material given in divided doses daily."

Royal Victoria Infirmary
Farnborough-Surrey, England

105. ANGLÉSIO D. *Nine cases of pernicious anemia treated with vitamin B₁₂*, *Minerva med.* 8: 802, 1950 (abstr. *Schweiz. med. Wchschr.* 80: 1125, Oct. 14, 1950)

Nine patients with pernicious anemia were treated with vitamin B₁₂. Improvement in the patients' conditions was evident in 48 to 60 hours. The reticulocyte crisis occurred on the seventh day after which there was rapid improvement in the blood picture. The daily dosage of vitamin B₁₂ was 2 or 3 mcg. and the total dose was 15 mcg. The treatment was repeated after a short interval.

106. QUERREX AND MINOR NOTES: *Pernicious anemia*, *J.A.M.A.* 150: 1058, Nov. 8, 1952.

Question "Please suggest treatment for a patient with true pernicious anemia in whom sensitivity to injections of vitamin B₁₂ in both the concentrate and crystalline forms, has developed. This is manifested by a generalised urticaria immediately following an injection. Preparations of vitamin B₁₂ and folic acid for oral administration will not maintain her

Answer "Sensitivity reactions to vitamin B₁₂ are rare. Since vitamin B₁₂ is a physiological agent, it is more likely that the patient is sensitive to the vehicle in which vitamin B₁₂ is dissolved, and it may be worthwhile to try products of several different manufacturers. The urticaria may also be checked by giving epinephrine (adrenalin®) in all one hour prior to the injection of vitamin B₁₂ or antihistamines orally two hours prior to the injection. In some cases, 50 mg. of cortisone, given a few hours prior to administration of vitamin B₁₂, may be effective. Cortisone could probably be given without any danger of side-effects, since vitamin B₁₂ would have to be administered only twice a month as a maintenance dose. Aside from that, desensitization to the injection of vitamin B₁₂ preparations, similar to that used in liver extract therapy may be attempted. Very small amounts of the vitamin B₁₂ preparation may be first injected intradermally and successive ones subcutaneously or intramuscularly. Thereafter the final dose is repeated daily for three days and then weekly for six weeks, after which maintenance therapy with single injections every two weeks is given. In some cases, parenteral administration may have to be abandoned completely; in such cases, powdered stomach (ventriculin®) in doses of 80 gm. per day may be tried, with or without antihistamines."

107. FLOOD F. T., and LIMARZI, L. R. *Follow-up study of 310 cases of pernicious anemia*, *J. Lab. & Clin. Med.* 36: 823-824, Nov. 1950 (in Soc. Proc.)

Three hundred ten patients with pernicious anemia were followed in the hematology clinic for periods up to 17 years. Bizarre granulocytes appeared earlier and persisted longer after liver therapy was started. There were 5 relapses in patients on maintenance doses of liver. Two of these patients were apparently receiving inactive liver since they recovered on the same dose of another preparation. Three were receiving liver at irregular intervals and recovered on recommended doses. One patient who showed a slow decline in red blood cells and hemoglobin on liver therapy gradually recovered on 15 mcg. of vitamin B₁₂ weekly. Three patients were sensitive to liver.

To study the natural history of pernicious anemia, 43 patients without neurologic changes and with hemoglobin above 12 Gm. were given normal saline instead of liver and followed by periodic neurologic and hematologic examinations. Two patients developed cord changes after 17

and 26 months respectively; the remainder stayed in remission for 3 to 72 months—14 of them for more than 2 years. None of the patients had free hydrochloric acid on gastric analysis before or after prolonged and intensive liver therapy.

Chicago, Ill.

108. MEYER, L. M., SAWITSKY, A., FINK, H., RITZ, N. D., and KRIM, M.: *Treatment of pernicious anemia with crystalline vitamin B₁₂*. Proc. Soc. Exper. Biol. & Med. 75: 366-367 Nov 1950.

Four patients with pernicious anemia in relapse were treated with 1 mcg. of crystalline vitamin B₁₂ daily for 10 days, and thereafter with 14 mcg. every 14 days. In all 4 cases the reticulocyte response was suboptimal and the blood count remained stationary in 3 cases and reached normal levels only after 103 days in the fourth.

A second similar group of 4 received 2 mcg. of vitamin B₁₂ daily for 10 days, then 14 mcg. at weekly or bi-weekly intervals. All 4 showed satisfactory clinical improvement, optimal reticulocyte responses, and normal blood levels in 48 to 69 days. Persistent signs of neurologic disease were observed in all 4 cases, but the symptoms disappeared in 1 of them.

New York University College of Medicine
New York, N. Y.

109. MEYER, L. M., SAWITSKY, A., COHEN, B. S., KRIM, M., and FADEN, R.: *Oral treatment of pernicious anemia with vitamin B₁₂*. Am. J. M. Sc. 220: 604-609 Dec. 1950.

Seven cases of pernicious anemia in relapse were treated with vitamin B₁₂ given orally in daily doses ranging from 75 to 300 mcg. Five of the patients had a clinical remission and in 1 patient who had symptoms of neurologic disease prior to treatment these symptoms disappeared. Three of the 5 patients showed normal hematologic values. In the remaining 2, they were subnormal. One of the latter patients had a severe concurrent pulmonary infection. This patient had a complete hematologic remission when treated with intramuscular vitamin B₁₂. One case had a maximal reticulocyte response, 3 had subnormal responses, and in the fifth patient no reticulocytosis occurred. The remaining 2 of the 7 patients did not respond to daily oral doses of 150 and 250 mcg. of vitamin B₁₂. They later responded to parenteral vitamin B₁₂ therapy.

In 1 patient, increasing the daily dose of B₁₂ to 500 mcg. raised hemoglobin and erythrocytes considerably, subsequent nausea and vomiting, however necessitated reduction of the dose. This side reaction was believed to be due to the large number of tablets and the amount of water ingested rather than to an effect of B₁₂ itself.

It is concluded that there is apparently no clearcut universal oral dose of vitamin B₁₂ for the treatment of pernicious anemia, that the deficiency of intrinsic factor in pernicious anemia patients is not always complete, and that a variable degree of absorption and utilization of B₁₂ takes place in some cases.

Columbia Memorial Hospital, and
Belmont Hospital
New York, N. Y.

110. LEAVELL, B. S., SHOTTON, D., and CROCKETT, C., JR.: *Pernicious anemia: diagnosis and treatment*. Virginia M. Monthly 77: 635-642, Dec. 1950.

At the University of Virginia Hospital the incidence of pernicious anemia is 92 per 100,000 white admissions and 66 per 100,000 colored admissions.

Liver extract, folic acid and vitamin B₁₂ are the most important preparations for the treatment of pernicious anemia. All of these agents produce a change from a megaloblastic erythropoiesis to the normoblastic type in about 72 hours.

Vitamin B₁₂ is the most potent, weight for weight, of the three preparations. One microgram of vitamin B₁₂ is about equivalent to one U.S.P. unit of liver extract. Whether vitamin B₁₂ equals or surpasses liver extract in the treatment of the neurological aspect of pernicious anemia remains to be established. Preliminary observations suggest that the vitamin is quite effective in this respect. In several patients with moderately severe neurologic involvement that have been treated with vitamin B₁₂, the authors noted that improvement seems to have been quicker and greater than is usually obtained with liver extract.

Folic acid is effective in producing a hematologic response in pernicious anemia and related macrocytic anemias, but it does not afford protection against the neurologic involvements of pernicious anemia. Its use in pernicious anemia is restricted to patients who develop hypersensitivity to liver extract and to those who have no neurologic manifestations.

University of Virginia School
Charlottesville, Va.

111. LECOCQ, R.: *Mise en évidence de l'action synergique de l'acide folique et de la Cobalamine (Vitamine B₁₂) dans la phase pseudo-irréversible de l'anémie pernicieuse (Evidence of the synergistic action of folic acid and Cobalamine [vitamin B₁₂] in the pseudo-irreversible phase of pernicious anemia)*. Thérapie 5: 85-90, 1950.

Excellent experimental results have been obtained with B₁₂ by English investigators in megaloblastic, gravid or puerperal anemia before the phase of pseudo-irreversibility. When given too late, vitamin B₁₂ has little effect, but is stored in the liver and when folic acid is administered later the reserves of vitamin B₁₂ permit the manifestation of synergic action, and the folic acid exerts action together with vitamin B₁₂, which the latter could not exert alone.

In association with Charchard and Mazoue, the author has been able to demonstrate a terminal phase of pseudo-irreversibility which explains the clinical failure of folic acid and of vitamin B₁₂. It should not be forgotten that these two factors differ not only in the active doses but that they constitute two vitamin elements capable of replacing each other in early treatment of megaloblastic anemia; in the terminal phase they should be used together because they have a particularly efficacious synergism.

High doses of this combination may cause disturbances of the leukocyte component which adenine (vitamin B₄) corrects advantageously.

112. KLIMA, R., and WENGRAF G: *Effect of vitamin B₁₂ in 34 cases of pernicious anemia*, Wien. med. Wochenschr 101 7 Jan. 6, 1951 (abstr. J.A.M.A. 1801 1302, April 21, 1951)

Twenty-six patients with pernicious anemia were treated with pure vitamin B₁₂. A daily dose of 15 mcg. of vitamin B₁₂ given intramuscularly or intravenously was adequate for restoration of a normal blood picture. Reactions of hypersensitivity or intolerance to vitamin B₁₂ did not occur. Mild symptoms of funicular myelitis, present in some patients were improved by vitamin B₁₂. Maintenance treatment with 15 mcg. per week of vitamin B₁₂ was given to 8 patients for about 10 months after the anemia was corrected, and the improvement was maintained, 7 of the patients improving further. Comparison of the results of vitamin B₁₂ therapy with those of folic acid and liver extract therapy showed that with sufficiently high doses an optimum hematopoietic effect can be obtained with each of the three methods. Folic acid has the advantage over vitamin B₁₂ in that it can be given orally in the same dosage as is required for parenteral administration. When vitamin B₁₂ is given by mouth, 400 mcg. are required to give the same effect as 15 mcg. given intramuscularly. Combined preparations of vitamin B₁₂, folic acid and liver extract were given to 8 patients. These preparations did not seem to show mutual potentiation of effect. Vitamin B₁₂ had a favorable effect on refractory nonregenerative anemia in some cases. Genuine aplastic anemia did not respond to vitamin B₁₂.

113. MILLS, J., and HEMSTED E. H. *Vitamin B₁₂ in pernicious anemia*, Lancet 1 237 238, Jan. 27 1951 (in Letters to the Editor)

Results are reported for a group of 144 pernicious anemia patients treated with vitamin B₁₂ for a year. The patients had previously been on controlled maintenance therapy with various liver extracts. Monthly intramuscular injections of vitamin B₁₂ were given, 37 patients receiving 20 mcg., 49 receiving 40 mcg., and 58 receiving 80 mcg. per month. The highest average red cell levels occurred in the group given 80 mcg. per month; 98 per cent of these patients showed red cell levels above 4,500,000 per cmm.

"When the condition of these patients treated with vitamin B₁₂ is compared with their previous record under liver therapy it is found that 3% show slightly lower red-cell levels on vitamin B₁₂ than on liver extract, 82% show no significant difference, and 65% are distinctly better on vitamin B₁₂ than they were on liver therapy. Thus a majority of our patients are distinctly better when treated with vitamin B₁₂ than they were on liver therapy even though, as shown above, more than half the patients are receiving less than an optimal dose of vitamin B₁₂. In the group receiving 80% of B₁₂ each month, 80% show a higher red-cell level than they did on liver therapy."

"From these observations, therefore, it would appear advisable to give at least 80% of vitamin B₁₂ at monthly intervals to maintain a red-cell level of 5,000,000 cmm., and it is possible that a larger dose will give still better results. There is no doubt in our minds that these patients have been better in every respect since vitamin B₁₂ replaced liver therapy."

Royal Brompton Hospital
London, England

114. CHALMERS J N M. *Haemopoietic activity of vitamin B₁₂ and B₁₂ in pernicious anemia*, Brit. M. J 1 161 164, Jan. 27 1951.

Nine cases of pernicious anemia in relapse were treated with crystalline vitamin B₁₂, and 5 cases with crystalline vitamin B₁₂. Both substances were given intramuscularly in a single dose of 20 mcg. Both produced pronounced clinical improvement, with considerable relief of glossitis and lessening or disappearance of subjective neurologic symptoms. One patient, who had signs of associated iron deficiency showed progressive clinical improvement after a course of vitamin B₁₂ given in a dosage of 40 mcg. every two weeks together with a course of oral ferrous sulfate. Normal blood values were attained in two months.

The author states that it is too early to draw conclusions however cases under investigation are showing progressive clinical and hematologic improvement on doses of 20 mcg. of vitamin B₁₂ given every two weeks.

St. George's Hospital and Medical School
London, England

115. REID C C K. *Two cases of pernicious anemia treated with vitamin B₁₂*, Brit. M. J 1 164-165, Jan. 27 1951

Two cases of pernicious anemia, 1 with early signs of subacute combined degeneration of the cord, were successfully treated with vitamin B₁₂ in the very small dosage of 10 mcg. In both cases the antianemic activity of B₁₂ was of the same order as that of crystalline vitamin B₁₂. In one case 10 mcg. of vitamin B₁₂ gave the same response as 1 cc. of extract, a liver extract having full antianemic activity at that dose level.

Westall General Hospital
Birmingham, England

116. EISENBERG S E. *The treatment of pernicious anemia*, Connecticut M. J 15 45-47 Jan. 1951.

Up to the present time, intramuscular administration of concentrated liver extract has been the treatment of choice for true Addisonian pernicious anemia. Now it seems likely that vitamin B₁₂ will take the place of liver extract. When administered intramuscularly 15 mcg. of vitamin B₁₂ has the same effect as 15 units of liver extract. An average dose of 1 mcg. a day of vitamin B₁₂ affects favorably the anemia, lesions of the mucous membranes, and the neurologic manifestations of pernicious anemia. It is the only pure chemical substance known which is effective in relieving subacute combined degeneration in persons with pernicious anemia.

Given orally vitamin B₁₂ is ineffective unless normal gastric juice of stomach or intestinal extracts are also given. By this means, 5 mcg. a day has produced a therapeutic response.

Folic acid is contraindicated because it does not benefit the manifestations of subacute combined degeneration, and may even have an unfavorable effect on the neurologic manifestations.

New Britain, Conn.

117. HAUSMANN K. *Haemopoietic effect of thymidine in pernicious anemia*, Lancet 1 829-830, Feb. 10, 1951.

The author describes the treatment of 2 cases of Addisonian pernicious anemia with a preparation made from purified liver extract and containing 85 to 90 per cent of thymidine. The average daily dose was 200 (100 to 250) mg. The total dosages were 2 and 2.9 Gm., respectively. These amounts exerted a hematopoietic effect comparable to that of corresponding doses of vitamin B₁₂, folic acid or thymine. The thymidine supply was exhausted after 16 days of treatment. The administration of vitamin B₁₂ in doses of 25 and 50 mcg. produced no second reticulocytosis.

The author does not expect thymidine to be of practical value in the treatment of pernicious anemia. He states, however, that the hematopoietic effect of thymidine supports the assumption that vitamin B₁₂ exerts an enzymatic effect on nuclear metabolism.

*Abt. Intern. Krankenhaus St. Georg
Breslau, Germany*

118. LICHTMAN H., GINSBERG V., and WATSON J: *Therapeutic effect of aureomycin in pernicious anemia*, Am. J. Med. 10: 239 Feb. 1951 (in Soc. Proc.)

In order to study the effect of alteration of the bacterial flora on pernicious anemia in relapse, preliminary studies were made on the effects of aureomycin in 3 cases of pernicious anemia and 1 case of nutritional macrocytic anemia. In the first case of pernicious anemia after a negative control period of 10 days on 3 mcg. daily of vitamin B₁₂, orally the addition of 3 Gm. of aureomycin daily resulted in a reticulocyte peak 17 days later. The red blood cells, hemoglobin, and the hematocrit increased in 37 days. In the second case, after a negative control period of 12 days on a diet free of animal protein plus 2 Gm. of aureomycin a day the addition of 200 Gm. of chopped beef daily resulted in a hematologic response. In the third case, 2 Gm. of aureomycin and 2 Gm. of streptomycin orally each day resulted in a suboptimal but significant hematologic response.

The patient with nutritional macrocytic anemia was treated with a diet free of animal protein and 2 Gm. of aureomycin daily for 13 days with no appreciable effect when 3 mcg. of vitamin B₁₂ was given by mouth daily a reticulocytosis and rise of red cells occurred. In each case the sternal marrow which was originally megaloblastic, became normoblastic.

*Kaiser County Hospital
Stockton, N. Y.*

119. ELLISON R. R., WOLFE, S., LICHTMAN, H., GINSBERG V., and WATSON J: *Effect of citrovorum factor in pernicious anemia*, Proc. Soc. Exper. Biol. & Med. 76: 366-370 Feb. 1951

Citrovorum factor has been shown to produce adequate hematologic and clinical response in 4 of 6 patients with Addisonian pernicious anemia and a submaximal response in another. Treatment was ineffective in the sixth patient.

120. QUELIES AND MINOR NOTES: *Folic acid and pernicious anemia*, J.A.M.A. 146: 299 May 19 1951

Question "Does folic acid administered by muscle or by mouth predispose to cord damage in pernicious

anemia? If folic acid and vitamin B₁₂ are given together per os, is this danger averted? Would it be advisable to administer folic acid and vitamin B₁₂ per os in patients with large subtotal gastrectomy in order to prevent development of pernicious anemia?

Answer "Folic acid whether administered orally or intramuscularly fails to protect against the development of cord damage and may predispose to the development of that condition in pernicious anemia. If vitamin B₁₂ is administered orally in large enough doses it will protect against this complication, but it is much more effective given intramuscularly. Neither folic acid nor vitamin B₁₂ should be administered routinely in patients with large subtotal gastrectomy. It would seem better practice for one to perform blood studies at suitable intervals to see whether a hyperchromic anemia was developing before instituting treatment."

121. CASTLE, W. B. *Present status of the etiology of pernicious anemia*, Ann. Int. Med. 34: 1093-1106, May 1951.

Authors summary "The information presently available suggests that Addisonian pernicious anemia is the clinical result of a deficiency chiefly of vitamin B₁₂. This deficiency is predominantly due to a lack of gastric secretion, itself usually a manifestation of hereditary predisposition and of advancing age. In some patients, local disease or surgical ablation destroys this essential function of the stomach. The consequent failure of vitamin B₁₂ to be assimilated adversely affects hematopoiesis and frequently also the integrity of the alimentary tract and of the nervous system. The proximal cause of the macrocytic anemia is both decreased production and increased destruction of defective red cells. Either both processes must be of only moderate degree, or the magnitude of each must be inversely related to that of the other.

Defective diets and intestinal dysfunction or disease, especially when gastric secretion is not significantly disturbed, may result in nutritional macrocytic anemia in which the predominant deficiency is not of vitamin B₁₂ but of pteroylglutamic acid. Dietary deficiency of ascorbic acid probably restricts the formation of the metabolically active and closely related form of pteroylglutamic acid known as the citrovorum factor or folic acid. Vitamin B₁₂, pteroylglutamic acid and ascorbic acid are apparently required for normal hematopoiesis. The full significance of their interrelations with respect to the nucleoprotein metabolism of microbes, of animals and of man remains to be disclosed."

Source, Mass.

122. KLEINSORGE, H., and KLUMBIES G: *Vitamin B₁₂ in treatment of pernicious anemia*, Med. Wch 20: 740. June 2, 1951

123. JARROLD T., HERRIGAN D., THOMPSON C., and WILTER, R. W. *The hematologic effect of folic acid (citrovorum factor) in persons with pernicious anemia*, Science 113: 688-689 June 15, 1951.

The authors cite studies from which the following deductions were made: 1) folic acid is a precursor of the citrovorum factor and ascorbic acid may play a part in

the conversion; 2) citrovorum factor and folic acid are probably identical; 3) folic acid may be a biologically important intermediate in the metabolism of folic acid; and 4) folic acid may be the metabolically active form to which folic acid is converted in persons with pernicious anemia in relapse before it exerts its hematopoietic effect.

In the present study 3 patients with pernicious anemia in relapse were treated with folic acid given intramuscularly for 10 consecutive days. The daily dose in 2 of the subjects was 3 mg. and in the other 1.5 mg. Hematologic responses occurred in all 3 patients. Results were as good as, but no better than, those which would be expected with similar amounts of folic acid. One of the patients who responded to the daily administration of 3 mg. of folic acid had previously failed to respond to 0.6 mg. daily for 10 days.

An additional patient who had relapsed hematologically while receiving 20 mg. of folic acid daily did not respond to a 10-day course of citrovorum factor 8 mg. daily. Subsequently he showed a desultory response to vitamin B₁₂ 15 mcg. daily for three weeks, then 20 mcg. daily.

In another part of this study citrovorum factor was instilled directly into the bone marrow cavity of 3 patients with pernicious anemia in relapse in doses of 0.06 mg., 1.5 mg., and 3 mg., respectively. In no instance was the erythrocyte maturation effect that follows similar administration of vitamin B₁₂ observed. Folic acid acts like folic acid in this respect.

The authors conclude from these results that folic acid, or citrovorum factor is a potent hematopoietic agent in pernicious anemia in relapse but it is no more effective than a similar dose of folic acid. Moreover this agent, like folic acid, must be altered in the body in a site other than the bone marrow cavity before it becomes active in hemopoiesis.

University of Cincinnati
Cincinnati, Ohio

- 124 REISNER, E. H., JR., and WEINER, L.: *Observations on the mechanism of the synergistic action of oral vitamin B₁₂ and folic acid in pernicious anemia*. Bull. New York Acad. Med. 27: 391, June 1951

Mayer and his associates attributed the synergistic action of vitamin B₁₂ and folic acid when given together to an intrinsic factor-like action of folic acid, with enhanced absorption of vitamin B₁₂. Since folic acid is not the intrinsic factor of Castle, the authors sought some other explanation. Intrinsic factor does not affect absorption of vitamin B₁₂ unless it is given within three hours of the latter but in 3 patients the authors found that 1 mg. or 0.67 mg. of folic acid increased absorption of 10 mcg. of vitamin B₁₂ when given 12 hours after administration of B₁₂. In one instance, giving the two substances simultaneously following the initial response gave no secondary reticulocyte activity which suggests that the effect is not the direct action of one substance upon the other in the gut.

One patient was given 10 mcg. of vitamin B₁₂ orally for a week, and 200 mcg. of folic acid parenterally for a

week, and then both substances. The reticulocyte activity was increased when the drugs were given concurrently proving that synergy is not due to enhanced absorption of B₁₂. While the effect may be additive, the authors believe that it is due to "the catalytic action of minute amounts of B₁₂ that are absorbed despite the absence of intrinsic factor upon the freely absorbable, but sub-optimal dose of folic acid." Further observation will be needed to see if the small amount of vitamin B₁₂ is enough to prevent aggravation of spinal cord disease by the folic acid.

New York University Post-Graduate Medical School
New York, N. Y.

- 125 RITZ, N. D., MEYER, L. M., BRAHIN, C., and SAWITSKY, A.: *Further observations on the oral treatment of pernicious anemia with subminimal doses of folic acid and vitamin B₁₂*. Acta Haematol. 5: 334-338, June 1951

A synergistic effect is noted when B₁₂ and FGA are used.

- 126 JAMES, G. W., III, and ABBOTT, L. D., JR.: *Effectiveness of thiocyanate analog of vitamin B₁₂ in pernicious anemia*. Proc. Soc. Exper. Biol. & Med. 77: 416-418, July 1951

Vitamin B₁₂ has been shown to be a cyano-cobalt coordination complex and has been designated cyano-cobalamin. Vitamins B_{12a} and B_{12b} are identical, and represent the replacement of the cyano group by a hydroxy group in the vitamin B₁₂ molecule. This substance is designated hydroxy-cobalamin. The thiocyanate analog, in which the cyanide ion of vitamin B₁₂ is replaced by the thiocyanate ion, has been found to be micro-biologically equivalent to vitamin B₁₂ in the L. lactis cup assay but to differ from vitamin B₁₂ in titrimetric assay methods.

The present paper reports studies of the action of the thiocyanate analog of vitamin B₁₂ in 2 previously untreated patients with pernicious anemia in relapse. After an initial dose of 15 mcg., 5 mcg. was given daily parenterally until the peak of reticulocyte response was reached, after which 8 mcg. was given every other day. In each patient, maximum reticulocytosis occurred on the seventh day of treatment. The moderately severe neurologic symptoms in the older patient (age 85) did not respond well. Treatment with 75 mcg. of the thiocyanate analog during nine days of hospitalization, and a total of 225 mcg. over a period of 61 days produced no noticeable improvement in the neurologic symptoms. Vitamin B₁₂ was then given, since the supply of the thiocyanate was exhausted, and the patient reported some subjective improvement after 90 mcg. had been given. In both cases the early biochemical and hematologic responses were the same as those observed with the same dosage of vitamin B₁₂. Further evaluation of the neurologic response to the thiocyanate analog is necessary.

Medical College of Virginia
Richmond, Va.

- 127 DAS GUPTA, C. R., and CHATTERJEE, J. B.: *Pernicious anemia in Asiatic Indians*. Blood 6: 631-638, July 1951.

In describing 3 cases of pernicious anemia in Asiatic Indians the authors corroborate the prevailing idea regarding the low incidence of pernicious anemia in this group.

One of the 3 patients was treated with folic acid, and during therapy neurologic complications appeared for the first time. This is typical in pernicious anemia treated with folic acid.

In their discussion, the authors state that pernicious anemia is apparently a pure deficiency disease in which a vitamin B₁₂ deficiency is caused by a deficiency of intrinsic factor as a result of irreversible changes in the gastric mucosa. In nutritional macrocytic anemia, neurologic complications are absent because the presence of intrinsic factor assures the absorption of some vitamin B₁₂ to preserve the integrity of the nervous system. Since folic acid completely cures nutritional macrocytic anemia, this disease may be a manifestation of folic acid deficiency. The production by folic acid of an excellent hematologic response in pernicious anemia indicates that a disturbance of this vitamin may be an etiologic factor in this disease. In conclusion, it is stated that the respective roles of folic acid and vitamin B₁₂ in the pathogenesis of pernicious anemia and nutritional macrocytic anemia, and also the relationship of one to the other are yet to be clarified.

*School of Tropical Medicine, and
Consultant Hospital for Tropical Diseases
Calcutta, India*

128. CAMERON D. G., TOWNSEND S. R., and MILLS, E. S. Pernicious anemia. *J. Maintenance treatment with liver extract*, *Canad. M. A. J.* 65: 241-245, Sept. 1951.

This is a report of 63 patients treated for one to twenty years with liver extract. All but 3 (with complicating disease) were maintained in satisfactory hematologic remission.

Seven of fifty with objective neurologic disease at onset of observation underwent complete remission; no progression was noted, but rather improvement in the great majority of those with neurologic involvement.

Reports are presented for the purpose of comparison with a study in progress evaluating effect of B₁₂ therapy on a long-term basis.

129. STRAUSS, M. R., and BROKAW R.: *Adrenocortical function in pernicious anemia*, *New England J. Med.* 245: 796-802, Nov. 22, 1951.

Manifestations common to both Addison's disease of the adrenal glands and pernicious anemia are listed and discussed in an article citing 50 references.

The authors made eosinophil counts before and after injection of corticotropin in 7 patients with pernicious anemia. In all, 53 counts were made and the fall in eosinophils was greater than 50 per cent in only eight of the tests. In contrast, all of 5 patients with hypochromic anemia due to blood loss showed significant falls in eosinophil counts following injection of corticotropin. Five patients with pernicious anemia in remission who had shown no significant declines in eosinophil levels after

single doses of 25 mg. of corticotropin received 25 mg. every six hours for several days. Significant eosinopenia occurred in only 1 of the 5 patients. Three patients with pernicious anemia (who did not respond to corticotropin with eosinopenia) were given cortisone 100 mg. orally twice a day or every six hours for a few doses; all showed significant falls in eosinophil counts.

It has been observed that patients with pernicious anemia excrete below-normal amounts of 17-ketosteroids in the urine. The normal amount for males is 8 mg. of urinary 17-ketosteroids in 24 hours. Three male patients with pernicious anemia were studied and they excreted 2.4, 4.8, and 5.7 mg. of 17-ketosteroids in a 24-hour period before vitamin B₁₂ therapy was instituted. The excretion was recorded as 3.2, 6.9, and 8.4 mg. respectively within two to three weeks after the initiation of vitamin B₁₂ therapy. The following comments concerning the possible relationship of vitamin B₁₂ to adrenal function are made: "It seems possible that a deficiency of this substance (vitamin B₁₂) leads to functional impairment of the adrenal cortex or the anterior lobe of the pituitary gland, either as a result of the general debilitating effect of the deficiency or because vitamin B₁₂ is actually necessary for their proper function. That the adrenal rather than the pituitary gland is involved is suggested by the failure of prolonged ACTH administration regularly to induce eosinopenia. . . should it be determined that vitamin B₁₂ is necessary for proper adrenal function, the possibility arises that a metabolic antagonist to vitamin B₁₂ would be capable of inducing adrenocortical insufficiency. The idea of producing such a state as a therapeutic procedure in serious hypertensive and other disorders by such means is intriguing. If found to be beneficial it would presumably be much less hazardous than the present method of surgical extirpation of the adrenal glands."

*Cushing F. D. Hospital
Framingham, Mass.*

130. UNGLEY C. C. *Some current views on the origin of pernicious anemia and the absorption of vitamin B₁₂*, *Tr. New York Acad. Sc.* 14: 25-28, Nov. 1951.

*Royal Free Hospital
Northwick Road, England*

131. CAMPBELL, D. C., and HALL, B. E. *Maintenance therapy with vitamin B₁₂ for patients with pernicious anemia*, *J. Lab. & Clin. Med.* 38: 797, Nov. 1951 (In Soc. Proc.)

"Vitamin B₁₂ has now been used for more than three years in the treatment of patients with pernicious anemia. Some question has been raised with regard to its ability to maintain these patients in satisfactory remission. Beard and his associates, in a preliminary study of eighteen patients with pernicious anemia maintained with vitamin B₁₂ found the results to be unsatisfactory in seven patients. Mills and Hemstedt, after following for one year 144 patients with pernicious anemia maintained on vitamin B₁₂, recommended a dose of 80 µg. per month.

"We have had the opportunity of following thirty-two patients with pernicious anemia who have been maintained on vitamin B₁₂ given intramuscularly for from twelve to twenty-nine months. All patients fulfilled the requirements for the diagnosis of pernicious anemia

including a histamine-fast achlorhydria. Fifteen of the group had evidence of subacute combined degeneration of the spinal cord, and eight additional patients had evidence of peripheral neuritis as indicated by paresthesia of the extremities. We have grouped these data regarding the maintenance dose of vitamin B₁₂ both as to micrograms per day and as to total dose for the interval of administration. An attempt is made to correlate the patients' clinical, hematologic, and neurologic status with these data."

Rochester Min.

132. MEYER, L. M., and DIEFENBACH, W. C. L. *Pernicious anemia treated with citrovorum factor (Leucovorin): report of a case*, *Am. J. Clin. Path.* 21: 1054-1056, Nov. 1951.

Authors summary: "Oral administration for three months of 6.0 mg. of citrovorum factor to a patient with pernicious anemia induced a satisfactory remission, except that signs of subacute degeneration of the spinal cord appeared. Neurologic changes were reversed after this therapy was discontinued and vitamin B₁₂ was administered parenterally."

*Vitamin Administration Hospital
 Evanston, Ill.*

133. REIMER, E. E.: *Clinical experiences with vitamin B₁₂ therapy* *Wien. klin. Wchnschr.* 63: 941 Dec. 14, 1951 (abstr. J.A.M.A. 148: 1256, April 5 1952)

The abstract reads, in part: "Based on treatment of more than 100 patients with pernicious anemia with vitamin B₁₂, a dose of 120 to 180 µg. depending on the severity of the anemia and the associated neurological symptoms, may be required for recompensation. Several daily doses of 50 µg. seem to exert a better antianemic effect than one massive dose. In 50 patients subjected to continued treatment, a monthly maintenance dose of 40 µg. (in divided doses of 20 µg. every two weeks) was required to obtain erythrocyte counts of 4,000,000. Several

of these patients were treated with vitamin B₁₂ for more than two years without requiring increasing doses, while increasing doses of folic acid were required for maintenance. The subjective well-being of all the patients was pronounced, and increases in weight and in mental alertness were observed frequently. Larger maintenance doses up to two times the usual dose are indicated in elderly patients with hypoproteolemia or neurological manifestations. Fifteen patients with pernicious anemia of more than three years' duration whose blood values decreased markedly following discontinuation of the liver maintenance dose were subjected to a therapeutic trial with orally administered tablets containing 5 µg. of vitamin B₁₂, 1 mg. of folic acid, and 0.25 gm. of extract of stomach mucous membrane. Four tablets were given daily for four months. Although this follow-up is too short for definite evaluation of the maintenance therapy, three to four tablets stabilized the blood values and increased the red blood cell count to 4,000,000, which had never been reached previously. The subjective well-being of the patients was remarkable, and neurological manifestations did not occur. Fairly normal conditions of absorption are indispensable for oral treatment with vitamin B₁₂. Paren-

teral administration of vitamin B₁₂ is recommended in patients with intercurrent infections, hypoproteolemia, severe diarrhea, and neurological manifestations. In 10 patients with decompensated pernicious anemia and associated mild to severe herpes zoster highly satisfactory results with disappearance of the cutaneous manifestations and the neuritic complaints were obtained with four or five massive doses of from 30 to 60 µg. of vitamin B₁₂.

134. PAUL, J. T. *Treatment of pernicious anemia*, *Postgrad. Med.* 10: 508-510, Dec. 1951.

Treatment with liver extract and vitamin B₁₂ is briefly discussed. Patients with neurologic lesions require higher doses than others. If the vitamin is given orally alone, 30 to 60 times the amount of the parenteral dose is necessary unless the vitamin is mixed with normal gastric juice.

*University of Illinois College of Medicine
 Chicago, Ill.*

135. LUDWIG, L. *Untersuchungen zur bakteriellen Synthese von Vit. B₁₂ im menschlichen Magen-Darmkanal (Bacterial synthesis of vitamin B₁₂ in the human gastrointestinal tract)* *Klin. Wchnschr.* 45/46: 770-771, 1951 (abstr. *Schweiz. med. Wchnschr.* 82: 732, July 12, 1952)

The apparent findings of the experiments seem to be that fecal extracts from pernicious anemia patients have hematopoietic activity when tested microbiologically and when given parenterally to patients with pernicious anemia. The author concludes that intestinal bacteria are able to synthesize vitamin B₁₂ in patients with pernicious anemia, but the presence of proteins in the diet is necessary for this.

136. GALT, J., HUNTER, R. B., and HILL, J. M. *Pernicious anemia superseded by polycythemia vera: report of a case*, *Am. J. M. Sc.* 223: 61-64, Jan. 1952.

A case history is described in which a patient with pernicious anemia was treated with vitamin B₁₂ and crude liver extract remission occurred. About a month after an acceptable hemoglobin level was reached the patient developed pain, swelling and redness of the right leg, which were diagnosed as symptoms of erythema. There was a slow but total remission on bed rest and penicillin therapy but it did not last. Six months after the remission from pernicious anemia it was apparent that the patient had polycythemia vera.

In discussing the unusual circumstance of a patient's having both pernicious anemia and polycythemia vera, the authors state "Speculation at the present time in regard to this unusual association of these diseases has little basis in known facts. It has been suggested, however that when they do co-exist, the basic stimulus for medullary over-production prevailing in polycythemia is present but that the nutrient factor involved in clinically established pernicious anemia would hinder any polycythemic response. Only upon administration of the missing anti-anemic factor could the polycythemia then emerge.

"One could surmise that excessive amounts of specific anti-anemic therapy might over-stimulate the marrow into producing an overt polycythemic state. Except for the occasionally dissimilar circumstance of a slightly over reactivity response previously mentioned, there is nothing to substantiate this.

"Some investigators have theorized that primary polycythemia is the antithesis of pernicious anemia with the over production of cells being due to excessive formation of hematopoietic factor. Experimental attempts to demonstrate this have not been convincing."

*Baylor University Hospital
Dallas, Texas*

- 137 GLASS, G. B. J., BOYD L. J., RUBINSTEIN M. A., and SVIGALS C. S. *Treatment of pernicious anemia by oral administration of vitamin B₁₂ and glandular mucoprotein recovered from gastric juice of humans* Am. J. Med. 12: 109 Jan. 1952 (In Soc. Proc.)

Eight patients with pernicious anemia in relapse were given 7 to 30 mcg. of vitamin B₁₂ daily and after a control period 50 to 200 mg. of glandular mucoprotein obtained from gastric contents rich in this substance was added. The results varied, but the general improvement in response when mucoprotein was given suggests that Castle's intrinsic hematopoietic factor is contained in or is identical with the glandular mucoprotein of the human gastric juice.

*New York Medical College,
Tupper and Park Avenue Hospitals, and
Westchester Hospital, Westchester, N. Y.
New York, N. Y.*

138. STURGIS, C. C. *Etiology and treatment of pernicious anemia*, Postgrad. Med. 11: 83-89 Feb. 1952.

Development of red blood cells in the bone marrow is now believed to depend on two dietary constituents, namely vitamin B₁₂ and folic acid. Vitamin B₁₂ is absorbed from the stomach as a result of the action of the intrinsic factor of Castle in the gastric secretion, is then stored in the liver as the erythrocyte maturing factor and is released as needed to control the rate of maturation of erythrocytes in the bone marrow. Folic acid is present in the food in the conjugated state. In the body it is split into free folic acid and changed by enzyme action into folic acid, which also functions to control the rate of maturation of red blood cells. Pernicious anemia is thought to be caused by a deficiency in the intrinsic factor in the gastric juice which impairs the absorption of vitamin B₁₂, creating a deficiency in this vitamin. For a time, folic acid continues to control the maturation of the red blood cells, but eventually the increased demand for folic acid produces a deficiency in this vitamin also, which causes delayed maturation of erythrocytes. It would be anticipated that administration of either folic acid or vitamin B₁₂ would be effective in controlling pernicious anemia. Usually the administration of vitamin B₁₂ to pernicious anemia patients in relapse produces an increased appetite which results in an increased intake of folic acid in foods. In a few patients administration of vitamin B₁₂ does not repair the deficiency of folic acid, and better results are obtained when folic acid is given in addition to vitamin B₁₂.

The bearing of this theory on the treatment of different types of macrocytic anemia is discussed. In general, macrocytic anemia will respond to folic acid in doses of 10 mg. daily but this is ineffective in the control of the neurologic manifestations of pernicious anemia, which respond to vitamin B₁₂. Practically all types of macrocytic anemia respond to vitamin B₁₂, with the exception of the macrocytic anemia of pregnancy in which folic acid is highly effective.

Three patients were presented to illustrate types of anemia and their treatment. One with pernicious anemia with neurologic manifestations had responded rapidly after a single intramuscular injection of 500 mcg. of vitamin B₁₂. Another had been maintained on liver therapy for 25 years. Neurologic involvement in this case had been present for only a few months when vitamin B₁₂ was started, and improved under liver treatment except for ataxia resulting from permanent injury to the spinal cord. Since the sole effective therapeutic component of refined liver extract is probably vitamin B₁₂, this vitamin is recommended, beginning with 20 mcg. intramuscularly every day for a week, and then at greater intervals. If, as occasionally happens, the increase in red blood count levels off at about 4,000,000 per cc., 5 mg. of folic acid should be given daily by mouth until the count is normal (4,500,000 for women and 5,000,000 for men).

The third patient was presented to illustrate macrocytic anemia not due to pernicious anemia, and to show the points to be considered in diagnosis. This patient had cirrhosis of the liver. The treatment recommended was folic acid with the addition, if this proved ineffective alone, of vitamin B₁₂ intramuscularly.

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Ann Arbor, Mich.*

- 139 SPRAY G. H., and WITTS L. J. *The utilization of folic acid given by mouth*, Clin. Sc. 11: 273-281, Aug. 1952 (abstr. J. Clin. Nutrition 1: 260 March-April 1953)

The increase in folic acid concentration in the plasma and in urinary excretion of this vitamin after oral administration of 1 mg. is much smaller in untreated pernicious anemia patients than in normal subjects. Treatment with liver extract or vitamin B₁₂ restores the response to oral folic acid. These results suggest that absorption of folic acid is impaired in pernicious anemia in relapse or that its utilization is increased. The authors favor the latter suggestion. Shortage of vitamin B₁₂ in pernicious anemia is supposed to increase the demand for folic acid.

- 140 PAVLOVSKY A.: *Foreign Newsletter—Argentina*, Blood 7: 478-480 April 1952.

Included in this communication is the following reference to treatment of pernicious anemia: "The same author has used vitamin B₁₂ orally and rectally with success. Consequently he revises the present concept of the importance of the deficiency in intrinsic factor in the pathogenesis of pernicious anemia. De Arzu has also confirmed the results of Meyer and his co-workers on the efficacy of the association of small doses of folic acid to vitamin B₁₂."

Buenos Aires, Argentina

141. TALLEY R. W., DOHERTY J. E., and SHUKERS C. F.: *Pernicious anemia complicated by acute granulocytic leukemia: a case report*, South. M. J. 45: 559-561, June 1952.

A patient who had been treated for pernicious anemia at intervals over four years developed acute granulocytic leukemia. At various times the pernicious anemia had responded to folic acid, to liver extracts, and to vitamin B₁₂. Neither liver extracts nor vitamin B₁₂ gave an erythrocyte or reticulocyte response after leukemia developed, and the patient died in about two months.

Four cases in which pernicious anemia and granulocytic leukemia coexisted are reported from the literature. This coexistence is rare, and is probably a coincidence. However potent liver preparations affect the production of granulocytes as well as the production of erythrocytes, and the possible role of liver extracts, folic acid, and vitamin B₁₂ in stimulating abnormal production of granulocytes should be investigated.

*Department of Arthropod School of Medicine
Little Rock, Ark.*

142. REISNER, E. H., and WEINER, L.: *Studies on the mutual effect of suboptimal oral doses of vitamin B₁₂ and folic acid in pernicious anemia*, New Eng. land J. Med. 247: 15-17 July 3, 1952.

Results of a study of the interrelation of folic acid and vitamin B₁₂ therapy in 4 patients with pernicious anemia are given. Folic acid was administered intramuscularly or orally in doses ranging from 1 to 0.15 mg. and vitamin B₁₂ by the same methods in doses ranging from 0.25 to 30.0 mcg. One patient responded completely to a daily oral dose of 1 mg. of folic acid, and another responded to 30 mcg. of vitamin B₁₂ given parenterally on two successive days, but not to parenteral folic acid (0.5 mg.) or oral folic acid (1 mg.) and B₁₂ (10 mcg.) combined. These 2 cases illustrate the varied response of pernicious anemia patients to folic acid.

In the other 2 patients, oral administration of 10 mcg. vitamin B₁₂, separated by a 12 hour interval from administration of 0.67 to 1 mg. folic acid, resulted in enhanced hematopoietic activity over that achieved with either drug alone. The dosage of each drug was suboptimal. These cases confirm the work of Meyer (*Am. J. Clin. Path.* 20: 454-457 1950) who suggested that folic acid acts like "intrinsic factor." But since the enhanced response is also found when the agents are given at 12 hour intervals, the authors state "Consequently the enhanced response is not due to increased absorption of vitamin B₁₂ from the gut, as is presumably the case with the intrinsic factor of gastric juice, but is probably the result of the additive or synergistic effects of the small amounts of B₁₂ that may be absorbed by at least certain patients with pernicious anemia and the readily absorbed folic acid."

*New York University Post-Graduate Medical School, and
Bellevue Hospital
New York, N. Y.*

143. MURPHY W. P.: *Twenty five years experience in treatment and management of pernicious anemia*, J.A.M.A. 149: 907-912, July 5, 1952.

The author traces the sequence of events that have gradually led to present-day recognition of signs and symptoms of pernicious anemia. Therapeutic agents, their specific uses and results of treatment are discussed.

In describing the work of the numerous investigators in the search for the active liver principle, the author refers to the research involving vitamin B₁₂.

"Subsequent work has supported the belief that vitamin B₁₂ is the factor or one of the factors of liver and its extracts that is effective in the treatment of pernicious anemia. The fact that it is effective when administered parenterally and is so only in much larger doses when ingested has led to the belief that it not only is the extrinsic factor of Castle but is actually the anti-pernicious-anemia factor. Hall observed that the response to orally administered vitamin B₁₂ in doses up to 100 times the minimal effective dose for parenteral administration was variable and unpredictable but that its effectiveness might be increased by providing a known source of intrinsic factor such as the gastric juice of normal human beings and extracts of the stomach and intestines of swine. The efficacy of ingested whole mammalian liver as a hemopoietic in megaloblastic anemias and for control of the central nervous system degeneration in those with associated achlorhydria is well known. Liver contains both folic acid and vitamin B₁₂. The quantity of vitamin B₁₂ present in therapeutically effective amounts of liver is less than the amount of the crystalline form that is effective when administered by the oral route. The quantity of folic acid present is also small, and, although it may be effective for hemopoiesis when ingested, it does not control the central nervous system changes. Meyer and his associates reported favorable results from the oral administration of vitamin B₁₂ combined with minimal amounts of folic acid. These and other data accumulating suggest that there is a cooperative action between folic acid (and/or its conjugates) and vitamin B₁₂ for the control of hemopoiesis in megaloblastic anemia and of central nervous system function in the presence of achlorhydria. Vitamin B₁₂ may act within the body to convert folic acid conjugates to folic acid so that they become available for hemopoiesis or it may enhance the utilization of folic acid for the protein metabolism of blood formation. In view of the rapidly accumulating knowledge of the mechanism of the effect of liver in the megaloblastic anemias, it may be desirable to revise the present concept of the nature of the intrinsic factor or the role it plays in the utilization of the anti-pernicious-anemia factor."

Included in the author's comment are the following references to achlorhydria: "Histamine-fast achlorhydria is the result of a congenital defect of the mucosa of the stomach and is probably present from birth in the person who at some time, presents other evidences of the disease. The well-recognized familial incidence of pernicious anemia should make it possible to anticipate the disease in other members of the families of these patients and to start preventive therapy in those with achlorhydria. The physician should be alert to the earliest signs and symptoms in all patients who present themselves for study."

"The recent demonstration by Segal and his associates of a method for determining the presence or absence of free hydrochloric acid in the stomach by means of an

ion-exchange resin, in place of the usual unpleasant method of gastric analysis, should simplify the problem of diagnosis. As both the physician and layman become more acutely aware of the benefits to be derived from liver and its extracts, early recognition and treatment should become practically universal."

The review is accompanied by 35 references.

McClintock St.
London, N.W.

144. BLACKBURN E. L., BURKE, J., ROSEMAN C., and WAYNE, E. J.: *Comparison of liver extract and vitamin B₁₂ (cyanocobalamin) in maintenance treatment of pernicious anaemia*, Brit. M. J. 2 245-248, Aug. 2, 1952.

Authors summary "Sixty patients suffering from pernicious anaemia have been maintained in good health on injections of vitamin B₁₂ (cyanocobalamin)

"Of these, 22 had been previously treated with liver extracts for relatively long periods and were then given vitamin B₁₂ for at least two years. Only one case showed a statistically significant decrease in the red-cell count. 11 cases had a significant increase. No case showed a significant decrease in haemoglobin concentration.

"The first 10 of the patients who had been treated with vitamin B₁₂ from the start of their illness showed significantly higher red-cell counts and haemoglobin concentrations when compared with 10 patients who received liver extract alone.

"A few patients were strikingly better on vitamin B₁₂ treatment; one relapsed because of too infrequent dosage.

"No evidence was found of sore tongue, gastrointestinal disturbance, neurological involvement, or leucopenia. Macrocytosis may persist.

"Vitamin B₁₂ injections are small in bulk, constant in potency, relatively cheap, and do not lead to sensitization. A maintenance dose of at least 50 µg. a fortnight is recommended for all patients with pernicious anaemia without neurological involvement."

University of Sheffield, and
Royal Infirmary and Hospital
Sheffield, England

145. BREWERTON, D. A., and ASHER, R. A. J. *The maintenance therapy of pernicious anaemia with vitamin B₁₂*, Lancet, 2 265-266 Aug. 9, 1952.

This is a report on maintenance therapy of pernicious anaemia with vitamin B₁₂ in 36 patients. Each has received at least 20 months therapy with vitamin B₁₂ and had previously been treated with liver preparations for at least a year. Dosage of vitamin B₁₂ ranged between 50 mcg. every four weeks to 100 mcg. every two weeks.

Subjectively 20 patients reported they felt better on vitamin B₁₂ than they had on liver. 13 felt no difference, and 3 felt worse. Of these latter one has a senile tremor which has been becoming more severe for five years; one has had severe subacute combined degeneration of the cord for eight years and her recent symptoms were relieved when she was given twice as much vitamin B₁₂ as she had been receiving; and the third was found on examination to be as well objectively as before, and review

of old records showed the same complaints had recurred many times in the past 10 years while she was receiving liver injections.

Clinical examination at the time of subjective evaluation showed no evidence of deterioration. In no case was the red-cell count below 4,000,000 per cubic millimeter for women or 4,400,000 for men. In commenting on vitamin B₁₂ the authors state: "It is cheap, relatively painless, and free from unpleasant reactions. Its potency is reliable."

Central Middlesex Hospital
London, England

146. REISNER, E. H., JR., and WEINER, L.: *Treatment of pernicious anemia with massive parenteral doses of vitamin B₁₂*, Bull. New York Acad. Med. 28 Aug. 1952 (In Soc. Proc.)

Urinary excretion studies on patients receiving injections of 100 to 1,000 mcg. of vitamin B₁₂ showed that 51 to 98 per cent of the injected dose could be recovered from the urine in the succeeding 72 hours. This seems to indicate that parenteral doses of more than 50 mcg. are largely wasted and therefore of no advantage over the smaller doses in the treatment of pernicious anaemia. In 14 patients with pernicious anaemia in relapse who were treated at weekly intervals with single injections of 1,000 mcg. of vitamin B₁₂, remissions of three to six months occurred in 7 and 4 had been in remission four, six, eight, and nine months when they were lost sight of. One patient did not show complete remission following the injection and developed an exacerbation of cord disease two months later. In 7 patients with chronic combined system disease due to pernicious anaemia, weekly injections of 1,000 mcg. of vitamin B₁₂ for periods of 4 to 18 weeks gave no improvement greater than would have been expected from conventional doses of vitamin B₁₂ or liver extract.

New York University Post-Graduate Medical School, and
Bellevue Hospital
New York, N. Y.

147. WALKER, W., and HUNTER, R. B. *Single massive dose of vitamin B₁₂ in untreated pernicious anaemia*, Brit. M. J. 2 593-595, Sept. 13, 1952.

Fifteen patients with untreated pernicious anaemia were given single 1 mg. intramuscular doses of vitamin B₁₂ and their progress was followed until relapse; no other anti-anemia therapy was given, except for oral iron when indicated. The clinical status and marrow closely corresponded with the blood counts, except that only one patient complained of lassitude when the red cell count fell below 4,500,000 (the criterion for relapse). The other patients still felt normally well at relapse.

Six of the patients have continued in remission after 84 to 358 days since their single injection of vitamin B₁₂. Seven of the patients who relapsed were given a second similar dose of vitamin B₁₂; 3 are still in remission six to twelve weeks later and the other relapsed 142 to 349 days after the second injection.

Neurologic symptoms disappeared, when they had existed, in all but one patient. This patient had paresthesia at the time of relapse following the first injection, condition disappeared after the second.

The authors state in their summary: "While of theoretical and practical interest, these findings do not justify without further trials, the routine treatment of pernicious anemia by 1 mg. doses of vitamin B₁₂ at long intervals."

*Dr. Andrew C. L. Green and
Mary E. H. Houghton
Dundee, Scotland*

148. CONLEY, C. L., GREEN, T. W., HARTMANN, R. C., and KREIVANS, J. R. *Prolonged treatment of pernicious anemia with vitamin B₁₂*, Am. J. Med. 13: 284-293, Sept. 1952.

Results of vitamin B₁₂ treatment of more than 100 patients with pernicious anemia are discussed.

Seven patients were given single intramuscular injections of 12.5, 25 or 100 mcg. of vitamin B₁₂ with no further therapy until the hematocrit stopped rising. In some cases the lowest dose produced a striking response, but 100 mcg. seemed to be necessary to produce a maximal hematologic effect. The general state of health usually improved within the first week. Neurologic response was variable. A single dose of 25 mcg. seemed to be more effective than 1 mcg. a day for 25 days.

Fifty four patients have been successfully maintained on a regular dosage schedule for more than one year; in 48 cases 45 mcg. has been given intramuscularly every six weeks, 2 patients have been given 150 mcg. every five months, 1 has received 150 mcg. every four months, 1 has received 30 mcg. every four weeks, and 2 have received 150 mcg. every four weeks.

No evidence has been found to support the theory that folic acid deficiency in addition to vitamin B₁₂ deficiency may exist in patients with pernicious anemia. All the patients in this series did well on vitamin B₁₂ alone. Administration of folic acid brings about a hematopoietic response but may permit development of severe subacute combined degeneration before adequate vitamin B₁₂ treatment is given. Ingestion of folic acid in multi vitamin preparations has sometimes resulted in the confusing picture of subacute combined degeneration in the absence of anemia. The authors state that folic acid has no place in the treatment of uncomplicated pernicious anemia although it does no harm in patients adequately treated with vitamin B₁₂. It is unnecessary in patients on normal diet.

Vitamin B₁₂ administered parenterally was as effective as liver extract and is preferable because the pure crystalline substance can be given in smaller volumes of solution and therefore causes less discomfort at the site of injection. Moreover hypersensitivity reactions are not uncommon after injection of liver extract, but single amounts of crystalline vitamin B₁₂ as large as 1,000 mcg. have often been given intravenously subcutaneously and intramuscularly without a single instance of undesirable effect. In addition, vitamin B₁₂ is less expensive than liver extract.

In 11 patients the administration of single oral doses of vitamin B₁₂ (1 to 10 mcg.) gave results comparable to those obtained with much smaller doses given parenterally. An attempt is being made to work out a schedule for oral treatment, but this is still in the experimental stage. One patient has been maintained for 20 months on 2 mg. of

B₁₂ by mouth every four weeks after the initial dose of 5 mg. (1 mg. every four weeks proved insufficient). Another patient has been maintained on 1 mg. every four weeks for 14 months, and 15 others are receiving 1 mg. orally every week, but the observation period is not yet long enough for evaluation of results.

In a group of patients with subacute combined degeneration, neurologic abnormalities of many years' standing were not affected by vitamin B₁₂ given parenterally in doses of 150 to 1,000 mcg. at intervals of one to four weeks over a period of five to seven months. Five of 6 patients with less severe neurologic manifestations improved during treatment with 150 mcg. of vitamin B₁₂ parenterally at intervals of two to four weeks for 8 to 29 months. Improvement was no greater than in other patients who received only 45 mcg. at six week intervals.

The thiocyanate derivative of vitamin B₁₂, MK-50, was used in one patient with pernicious anemia. Response to a single intramuscular injection of 25 mcg. was prompt, and the patient was maintained in complete remission by injection of 45 mcg. every six weeks for as long as the material was available (10 months). Since then the patient has been maintained on B₁₂.

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Baltimore, Md.*

149. BASTRUP MADSEN, P. *Deficiency of antipernicious anemia principle as cause of chronic glossitis without megalocytic anemia*, Nord. med. 48: 1444-1447, Oct. 17 1952 (abstr. J.A.M.A. 151: 523-524 Feb. 7 1953).

"Bastrup-Madsen reports on five patients in whom glossitis was regarded as a manifestation of latent pernicious anemia although megalocytic anemia was absent. The lingual symptoms had lasted from 6 months to 15 years before the start of specific antipernicious anemia treatment. The bone marrow showed maturation disturbances in the myeloid series of the type seen in pernicious anemia. In all cases improvement in the lingual symptoms occurred from 5 to 10 days after specific treatment was begun, with complete disappearance of the symptoms in one of the three cases in which liver treatment was given and in the two cases in which vitamin B₁₂ was given. The color index approached 1. In the four cases in which the bone marrow was examined after specific treatment for two weeks the bone marrow picture had returned to normal. As in every other form of pernicious anemia, specific treatment once started must be continued. Interruption of the treatment may lead to the development of irreparable tongue and nerve lesions."

150. MURPHY W. P., and HOWARD, L.: *A comparison of the effect of vitamin B₁₂ with that of liver extract in the treatment of pernicious anemia during relapse and for maintenance*, New England J. Med. 247: 838-840, Nov. 27 1952.

Clinical effects of vitamin B₁₂ and liver extract were found to be similar in pernicious anemia patients both during relapse and while on maintenance therapy. Of the patients treated during relapse, 8 received intramuscular injections of crystalline vitamin B₁₂, and 20 received liver extract. Reticulocyte levels in the vitamin B₁₂ treated group compared favorably with those in the group treated

with liver extract, though comparison of erythrocyte counts showed an almost uniformly greater increase at all initial levels in the patients treated with liver extract. The 8 patients who were treated with vitamin B₁₂ during relapse continued to receive it for maintenance therapy. All have remained in excellent health with erythrocyte levels within the normal range.

Nine patients who had previously been maintained on liver extract were changed to vitamin B₁₂, and have been observed for periods ranging from 16 months to three years. The average erythrocyte counts were higher for all patients on B₁₂ therapy than on liver extract therapy. One patient in the maintenance group was returned to liver therapy because of severe central nervous system involvement with locomotor difficulty which did not appear to be well controlled by vitamin B₁₂. Severe neural disturbance present in 2 other patients receiving vitamin B₁₂ did not progress. The authors suggest that 1 U.S.P. anti-pernicious anemia unit of liver extract is equivalent to 1.3 mcg. of vitamin B₁₂.

Peter Bent Brophers Hospital, and
Harvard Medical School
Boston, Mass.

151. SCHRUMPF, A. *Vitamin B₁₂ and folic acid in small doses orally in pernicious anemia*, Nord. med. 48: 1621, Nov. 1952.

152. THEDERING and RIETHMULLER: *Acido folico y vitamina B₁₂ en las anemias megaloblasticas (Folic acid and vitamin B₁₂ in megaloblastic anemias)* Folia Clin. Internacional 2: 571, 1952 (abstr. Orientacion med. 1: 805, June 26, 1953).

The combination of folic acid and vitamin B₁₂ in pernicious anemia produced results decidedly superior to those achieved with either drug alone. The results are most evident in long term maintenance therapy of pernicious anemia. The authors advise immediate use of this substance in all cases there is no danger in the use of the combination.

153. HECK, F. J. *Treatment of anemia*, Minnesota Med. 36: 40-44, Jan. 1953.

In a review of the types of anemias and their treatment, it is stated that for patients with pernicious anemia, "There is no reason to believe that vitamin B₁₂ will not do everything that a good potent liver extract will do and by substituting 15 micrograms of vitamin B₁₂ for the 15 units of liver extract, basically the same result will be obtained."

Mayo Clinic
Rochester, Minn.

154. REISNER, E. H., JR., and WEINER, L. *The treatment of pernicious anemia with massive parenteral doses of vitamin B₁₂*, Blood 8: 81-85 Jan. 1953.

Studies were undertaken to determine the effectiveness of single massive injections of vitamin B₁₂ in pernicious anemia in relapse and in stabilized cases of combined system disease. Fourteen patients with pernicious anemia in relapse received single intramuscular injections of 1,000 mcg. of vitamin B₁₂, followed by complete hematologic remissions ranging from three to seven months in

all but one case. Seven patients in hematologic remission with combined system disease received weekly injections of 1,000 mcg. of vitamin B₁₂ for periods ranging from four to thirteen weeks. Four patients showed no evidence of objective improvement and one patient resumed liver injections because she felt better on that therapy. One patient obtained continued improvement in cord disease, and another who had had symptoms of combined sclerosis for four years showed moderate improvement, which was not progressive, in gait and balance. The amount of vitamin B₁₂ recovered 48 hours after injection from the urine of the patients treated in relapse ranged from 51 to 98 per cent of the injected dose.

The authors draw the following conclusions: "These studies indicate that massive single injections of vitamin B₁₂ cannot be substituted for more frequent regular injection of smaller doses in the treatment of pernicious anemia, without the danger of relapse and the aggravation of central nervous system disease. Furthermore, there is no evidence to indicate that a greater degree of improvement follows the regular weekly injections of massive doses of vitamin B₁₂ to patients with chronic combined system disease than occurs with regular conventional doses. This is probably because amounts of B₁₂ above a threshold value of 25 to 50 μ g. by injection, are rapidly and quantitatively excreted in the urine."

Included in the discussion is the report of remarkable improvement in a previously intractable case of osteitis secondary to arthritis with daily doses of 1,000 μ g. of vitamin B₁₂ given intramuscularly. It is concluded that in such a case the mechanism of action of B₁₂ was different from that in combined system disease resulting from pernicious anemia.

New York University Post-Graduate Medical School, and
Bellevue Hospital
New York, N. Y.

155. BEACHAM, G. C., and HEINLE, R. W. *Maintenance therapy of pernicious anemia with vitamin B₁₂*, J. Lab. & Clin. Med. 41: 65-77 Jan. 1953.

Authors' summary: "Patients with pernicious anemia were given maintenance therapy with crystalline vitamin B₁₂, and a concentrate containing vitamin B₁₂ and vitamin B₁₂ for periods up to sixteen months. In the dosages used, these substances were as effective as purified liver extract.

"An average daily dose of 1 μ g. given intramuscularly at intervals of three or four weeks appeared to be sufficient to maintain normal erythrocyte levels and to prevent the development or progression of neurologic or lingual lesions.

"In previous experiments, the concentrate was less effective than the crystalline preparation, but with the concentrates available now there is probably no significant difference.

"Mild macrocytosis persisted in many of the patients and was not altered by the intramuscular administration of purified liver extract nor by the addition of daily oral doses of folic acid. Comparison of the treated patients with a series of normals from the literature indicates that the relationship of the erythrocyte level to value in the two series is different, even when patients have high normal blood values.

"This suggests the possibility that neither vitamin B₁₂ purified liver extract, nor folic acid given singly or in combination completely corrects the erythrocyte abnormality in pernicious anemia and suggests that some other substance(s) may be necessary in addition.

"From the practical standpoint, however treatment with sufficient amounts of vitamin B₁₂, B₁₂ concentrates, or purified liver extract maintains normal erythrocyte levels and prevents development or progression of neurological and lingual lesions."

Western Reserve University School of Medicine, and
University Hospitals
Cleveland, Ohio

155. FIELD R.: Cases from the Medical Grand Rounds (Cases 235 and 236) Am. Pract. 4 143-146, Feb. 1953.

Two cases of pernicious anemia are presented and discussed in detail. In one patient the reticulocyte count rose about 10 per cent on 100 mcg. of vitamin B₁₂ intramuscularly daily. However treatment was changed to liver extract since it was thought that the patient's response to B₁₂ was slow. Lack of striking response to either agent was ascribed to an active bladder infection. The author feels that one U.S.P. unit of liver is equivalent in 3 to 5 mcg. of vitamin B₁₂. Thus a maintenance dose is 30 U.S.P. units of liver extract per four weeks, or at least 100 mcg. of vitamin B₁₂.

Massachusetts General Hospital
Boston, Mass.

157. MONTRO R. W., REBUCK, J. W., and BRENNAN M. J. Crystalline B₁₂ inhalation therapy in pernicious anemia, Am. J. M. Sc. 225 113-119 Feb. 1953.

Crystalline vitamin B₁₂ given by inhalation to 3 patients with pernicious anemia caused adequate clinical and hematologic responses. Two patients received inhalations of vitamin B₁₂ in saline administered by pressure tank and a vaponephrin nebulizer. The third received vitamin B₁₂ and lactose powder by means of a dust inhaler. Two of the patients had severe and the other mild pernicious anemia. One had symptoms indicating an early stage of subacute combined degeneration of the cord.

The dosage schedule was similar to that used for parenteral therapy. The lungs were not adversely affected. Detectable amounts of B₁₂ activity were found in the urine after therapy.

In an addendum it is stated that 3 patients with pernicious anemia in relapse have been adequately treated by inhalation of crystalline vitamin B₁₂.

The material given by aerosol contained 15 mcg. of vitamin B₁₂ per cc. The dust mixture contained 1,000 mcg. of vitamin B₁₂ in 0.1 cc. of lactose powder.

Harvey Fund Hospital
Newark, N. J.

158. ØSTLING, G., NYBERG W., and GORDIN R.: Antianemic activity of alkali-treated crude liver extract, Acta med. Scand. 154 40-43, March 23, 1953.

In a clinically effective "crude" liver extract which contained 3.5 mcg. of vitamin B₁₂ per cc., folic acid, folinic acid and deoxyribosides in amounts too small to be clinically effective, and two microbiologically effective alkali stable factors, the vitamin B₁₂ was destroyed by boiling with alkali. After this treatment the liver extract was found to have the same therapeutic effect in pernicious anemia as extract which had not been treated with alkali. Three cases are reported, 2 of cryptogenetic pernicious anemia and 1 of pernicious anemia which may have been the result of tapeworm infestation.

Maria Hospital
Helsingfors, Finland

159. ANNOTATION Maintenance treatment in pernicious anemia, Lancet 1 732-733, April 11, 1953.

After a review of seven recent references to the successful use of vitamin B₁₂ in pernicious anemia, the annotation is concluded with this paragraph:

"Vitamin B₁₂ provides a safe and sure means of maintaining in good health patients with classical pernicious anemia. If it fails to give good maintenance, then complications must be sought or the diagnosis reconsidered. With vitamin B₁₂ and the standard liver extracts freely available, there should in our view be no need to use so-called complete haematinics. Folic acid is commonly one constituent of these and a patient with pernicious anemia who is given folic acid is exposed to the risk of affection of the central nervous system—a risk that is surely unjustifiable when safe treatments are in hand."

160. BOUCHER, R., and PROULX, A.: L'anémie pernicienne et son traitement: présentation d'un cas classique (Pernicious anemia and its treatment: presentation of a classic case) Union méd. du Canada 82 880-885, April 1953.

Good response to vitamin B₁₂ in a case of pernicious anemia is reported. The dosage was 15 mcg. a day parenterally. Alternating the B₁₂ with 15 units of calves liver did not hasten response. After the seventh week the dosage was reduced to 15 mcg. weekly. Two weeks later the patient insisted on leaving the hospital. She was advised to take 15 mcg. of vitamin B₁₂ every two weeks.

Hôpital St-Jacques
Montreal, Canada

161. MEYER, L. M., SAWITSKY A., RITZ, N. D., and BRAHIN C.: Treatment of pernicious anemia with crystalline vitamin B₁₂, Blood 8 358-369 April 1953.

Crystalline vitamin B₁₂ was given intramuscularly to 9 patients with pernicious anemia in relapse. A daily dose of 1.0 mcg. for 35 to 210 days gave an unsatisfactory clinical and hematologic response in 6 patients. In 3 patients the daily dose of vitamin B₁₂ was 2 mcg. for 90 to 190 days; satisfactory clinical and hematologic remissions were obtained. "Crystalline vitamin B₁₂ is probably less potent as an anti-anemia agent than crystalline vitamin B₁₂."

Columbia Memorial Hospital
Folger Island, N. Y.
Veterans Administration Hospital
Bronx, N. Y.

162. SAWITSKY A., MEYER, L. M., MCINERNEY R., and DIEFFENBACH, W. C. L.: Oral treatment of pernicious anemia with citrovorum factor (Leucovorin) *Acta med. Scandinav* 145 332-337 May 27 1953.

Five patients with pernicious anemia in relapse were treated with citrovorum factor in daily oral doses of 0.15 to 15.0 mg. One patient failed to respond and another responded but relapsed after two months; both these patients responded to intramuscular injections of vitamin B₁₂. Two patients showed suboptimal reticulocyte peaks but were not followed for long enough periods to determine the maximal hematologic effect. One patient attained normal blood levels and was in good condition six months after the institution of therapy. Symptomatic improvement accompanied hematologic improvement in each case.

Queens General Hospital
Jamaica, N. Y.
Yonkers Adjuvant Institute, N. Y.
Bronx, N. Y.

163. MEYER, L. M., MCINERNEY R., and RITZ, N. D.: Intravenous treatment of pernicious anemia with vitamin B₁₂. *J. Clin. Nutrition* 1 299-301 May June 1953.

Authors' conclusions: "Daily intravenous administration of 0.5 or 1.0 g. of vitamin B₁₂ to 5 patients with pernicious anemia in relapse induced a satisfactory clinical remission.

"Erythrocyte regeneration was satisfactory in 1 case (receiving 0.5 g. per day) and greater than anticipated in 4 cases (receiving 1.0 g. per day)

"Erythremic levels were attained in 2 patients treated for 41 and 87 days with 1.0 g. of vitamin B₁₂ daily"

Yonkers Adjuvant Institute, N. Y.
Bronx, N. Y.
Queens General Hospital
Jamaica, N. Y.

ANEMIAS IN

PREGNANCY PUERPERIUM

164. DAY L. A., HALL, B. E., and PEASE, G. L. Macrocytic anemia of pregnancy refractory to vitamin B₁₂ therapy response to treatment with folic acid: report of a case, *Proc. Staff Meet., Mayo Clin.* 24 149-157 March 30, 1949

A patient with macrocytic anemia of pregnancy was treated with vitamin B₁₂ after treatment with liver extract had been unsuccessful. Vitamin B₁₂ was started on the 24th day of illness and was given parenterally in a total amount of 27.5 mcg. over eight days. No hemopoietic response occurred. The patient became nauseated and vomited after each injection of vitamin B₁₂. She vomited all afternoon following the last injection and passed 15 watery stools.

On the 32nd day of illness, folic acid therapy was started. The initial dose was 50 mg. parenterally and each day thereafter (except for one day a week) 15 mg. of folic acid was given until delivery occurred one month later. Both the hemopoietic and clinical responses to folic acid were excellent.

Mayo Clinic
Rochester, Minn.

165. FURMAN R. H., DANIELS W. B., JR., HEFNER, L. L., JONES E., and DARBY W. J.: Pernicious anemia of pregnancy: failure of vitamin B₁₂, therapy successful treatment with folic acid: report of a case. *Ann. Practitioner & Digest of Treatment* 1 146-147 Feb. 1950.

A case of so-called pernicious anemia of pregnancy is reported which failed to respond to vitamin B₁₂ treatment (10 mcg. a day intramuscularly for a week). Folic acid treatment was then given in intramuscular doses of 15 mg. daily for 20 days; the patient responded well. Three similar cases have been reported in the literature.

The resemblance of this type of anemia to the tropical macrocytic anemia described by Wills has long been evident, and evidence is increasing for the identity of vitamin M, "Wills factor" and folic acid. The existence of a group of anemias with megaloblastic bone marrow which responded to folic acid but not to parenteral liver therapy or vitamin B₁₂ should be recognized.

Vanderbilt University School of Medicine
Knoxville, Tenn.

166. HOFMEISTER, F. J., STOFFER, J. G., and STEHLIN J. S. Pernicious anemia of pregnancy. *Wisconsin Med. J.* 49 129- Feb. 1950.

167. GINSBERG V., WATSON, J., and LICHTMAN H.: Megaloblastic anemia of pregnancy response to pteroylglutamic acid after failure of response to liver extract and vitamin B₁₂. *J. Lab. & Clin. Med.* 36 238-241, Aug. 1950.

The authors describe a case of megaloblastic anemia of pregnancy which failed to respond to refined liver extract or to vitamin B₁₂ in a dosage of 1 mcg. a day given intramuscularly for 13 days. No adverse reactions to vitamin B₁₂ were observed, and a severe glossitis cleared up after one week of therapy. The patient subsequently responded maximally to therapy with folic acid, which was given intramuscularly in a dosage of 15 mg. three times a week.

Emory County Hospital
Brockton, N. Y.

168. HOLLY R. G. Megaloblastic anemia in pregnancy. Remission following combined therapy with ascorbic acid and vitamin B₁₂. *Proc. Soc. Exper. Biol. & Med.* 78 238-241 Oct. 1951

Megaloblastic anemia of pregnancy is refractory to vitamin B₁₂. It may be related to ascorbic acid deficiency—all 5 cases reported here occurred in April and May. Determinations of plasma ascorbic acid levels were made in 3 of these patients; the values were decreased or zero. Three of the patients failed to respond to vitamin B₁₂ or to ascorbic acid alone, but a combination of the two agents gave a complete hematologic remission. The fourth patient responded to combined folic acid and transfusion therapy and the fifth had a spontaneous remission following delivery.

University of Massachusetts Medical School
Worcester, Mass.

169. CHAUDHURI, S.: Vitamin B₁₂ in megaloblastic anemia of pregnancy and tropical nutritional macrocytic anemia, *Brit. M. J.* 2 825-828, Oct. 6, 1951

Author's summary "Sixteen cases of megaloblastic anemia of pregnancy and the puerperium were treated successfully with parenteral vitamin B₁₂. Out of five cases of tropical megaloblastic anemia treated with vitamin B₁₂, four responded to vitamin B₁₂ and one responded better to subsequent folic acid therapy.

"Four micrograms of vitamin B₁₂ a day was the estimated requirement in mild cases of macrocytic anemia of pregnancy and 10 µg. in severe cases."

Lady Hardinge Medical College
New Delhi, India

170. WOODRUFF A. W. *Anaemia of pregnancy among Africans in Nigeria*, Brit. M. J. 2 1415-1423, Dec. 15, 1951

In an article concerning anemia of pregnancy among Africans it is reported that an injection of 80 mcg. of vitamin B₁₂ was given without response to a case of normocytic anemia. Folic acid failed also. Of 2 cases of macrocytic anemia given 120 mcg. and 100 mcg., respectively one responded slightly and the other not at all. A patient with macrocytic anemia who was given 100 mcg. of vitamin B₁₂ six days after delivery improved, but apparently no faster than those patients having natural remissions.

University College
Ibadan, Nigeria

171. FOY H., KONDI, A., and HARGREAVES, A.: *Response of the megaloblastic anaemias of pregnancy to animal protein factor: preliminary report*, Brit. M. J. 1 852-853, April 19 1952.

In a report of one case the authors suggest that "the response may have been due to (a) changes in the bacterial flora of the gut brought about by the antibiotic present in the A.P.F.; (b) an unknown haemopoietic factor present in the A.P.F. (c) the small amount of vitamin B₁₂ present in the A.P.F. or (d) sparing action of the aureomycin [in A.P.F.] on the vitamin B₁₂."

Williams Trust Research Laboratories, and
Colonial Medical Service
Kampala, Africa

172. O'GRADY J. W., RIVA, H. L., and RITZEN THALER, J. G. *Hemolytic anemia in pregnancy*, Am. J. Obst. & Gynec. 65: 1333-1344 June 1953.

Two cases of hemolytic anemia associated with pregnancy are reported. One patient was treated conservatively (transfusion, vitamin B₁₂ and folic acid). The second patient was treated by splenectomy. The first patient had a premature living child, the second a normal baby.

Robert Reid Army Hospital
Friedrichs, A. C.

173. PATEL, J. C., and KOCHER, B. R.: *Vitamin B₁₂ in macrocytic anaemia of pregnancy and the puerperium*, Brit. M. J. 1 924-927 April 22, 1950.

Five cases of macrocytic anemia of pregnancy and the puerperium were treated with vitamin B₁₂ by intramuscular injection. In all of the cases a 40-mg. dose produced moderately good to striking response which reached a maximum on the tenth day. A 20-mg. dose,

tried in 1 of the patients, produced partial improvement, both clinical and hematologic. The authors believe a further increase in the dosage would probably bring the blood picture to normal.

King Edward Memorial Hospital
Bombay, India

174. UNGLEY C. C., and THOMPSON R. B. *Vitamin B₁₂ and folic acid in megaloblastic anaemias of pregnancy and the puerperium*, Brit. M. J. 1 919-924, April 22, 1950.

In 6 patients with megaloblastic anemia associated with pregnancy or the puerperium, the injection of vitamin B₁₂ in doses of 65 to 80 mcg., had no effect. All the patients responded to the subsequent oral administration of folic acid, in daily doses of 2.5 or 20 mg. In one serious case a 80-mg. dose of folic acid was given intravenously.

Royal Victoria Infirmary and
King's College
Hammersmith-Spencer-Tyrie, England

175. THOMPSON R. B., and UNGLEY C. C. *Megaloblastic anaemia of pregnancy and the puerperium*, Quart. J. Med. 20 187-204, 1951 (abstr. Blood 6 1800-1801 Dec. 1951)

"This report includes observations on 45 patients during the past seventeen years, 26 of whom were followed for periods of from one to fourteen years. The clinical features were essentially those previously described by these and other authors.

"The response to therapy reported here further emphasizes the differences between megaloblastic anemia of pregnancy and Addisonian pernicious anemia. This observation is contrary to the theory that megaloblastic anemia of pregnancy is due to a deficiency of the anti-pernicious anemia principle, brought about either by lack of extrinsic factor from the diet or by a temporary absence of intrinsic factor from the stomach. All 8 patients who received folic acid responded. Responses were also obtained to raw liver pulp and to yeast extract.

"The dietary histories were investigated in 27 cases in only 11 of these could the anemia be explained on a deficiency basis.

"The survival rate of transfused normal erythrocytes was followed in three cases and in each there was an increased rate of elimination."

Royal Victoria Infirmary
Hammersmith-Spencer-Tyrie, England

176. EDITORIAL. *Folic acid deficiency in macrocytic anemia of pregnancy and infancy* J.A.M.A. 148 1422, April 19 1952.

The role of folic acid in the prevention and treatment of macrocytic anemia is discussed in an editorial with eight references. It has been demonstrated that there is a physiologic relationship between the failure of hemopoiesis in the mother and failure of hemopoiesis in the nursing infant due to a deficiency of folic acid. Lack of folic acid explains why certain patients did not respond to the administration of the purified anti-pernicious anemia liver extract. Most potent antianemia liver extracts

are rich in vitamin B₁₂ but are almost devoid of folic acid. Liver extract, vitamin B₁₂, and folic acid each fail in the treatment of some types of macrocytic anemia.

"Nothing takes the place of diagnostic acumen and appreciation of the activity and specificity of the various vitamins. The fact that folic acid does not relieve acute neural degeneration is not a general indictment against it, for its value in some types of macrocytic anemia is beyond dispute. Likewise the fact that vitamin B₁₂ does not always relieve the macrocytic anemia of pregnancy and the megaloblastic anemia of infancy is no indictment against it. Vitamin B₁₂, or substances that act similarly are essential for the integrity of the nervous system. Folic acid is essential in some types of macrocytic anemia; hence, both vitamin B₁₂ and folic acid are essential for life."

NEWBORN

- 177 ANDERSON D. E. *Chronic aregenerative anemia of the newborn*, *AL J Australia* 1 573-576, April 26, 1952 (abstr. *Am. J. Digest. Dis.* 20 88, March 1953)

Chronic aregenerative anemia of the newborn (chronic hypoplastic anemia, congenital hypoplastic anemia) is not benefited by folic acid, liver extract and vitamin B₁₂. Repeated blood transfusions permit the patient to live for many years but the ultimate prognosis is not yet known.

INFANCY

178. McPHERSON A. Z., JONSSON U., and RUNDLES, R. W. *Vitamin B₁₂ therapy in megaloblastic anemia of infancy* *J. Pediat.* 34 529-536, May 1949

Two infants with megaloblastic anemia have been successfully treated with vitamin B₁₂. One was a Negro aged 11 months. Two days after a single intramuscular injection of 0.002 mg. of vitamin B₁₂, her reticulocyte percentage rose and reached a peak of 69.1 per cent on the seventh day. A bone marrow examination made at this time showed a suggestion of megaloblastic development, but on the thirteenth day after injection another examination showed normoblastic cellular development with active cell proliferation. The regeneration of the blood was rapid. The child was discharged two weeks after the injection. Follow-up examinations 3 and 10 weeks later showed weight gain and normal blood values.

The other child, aged 7 months, was given 0.002 mg. of vitamin B₁₂ intramuscularly and a week later 0.005 mg. On the twelfth day the dosage was increased to 0.002 mg. daily and continued for 18 days. Serum albumin was given intravenously for four days. By the nineteenth day improvement was evident. The patient was discharged 32 days after the beginning of treatment and appeared perfectly well at a follow-up examination six weeks later.

- 179 WOODRUFF C. W., RIPPY H. W., PETERSON J. C., and DARBY W. J. *Variable response to vitamin B₁₂ of megaloblastic anemia of infancy* *Pediatrics* 4 723-729 Dec. 1949

Two infants with megaloblastic anemia showed a good hematologic response to vitamin B₁₂ (0.025 mg. given subcutaneously in one case and intramuscularly in the other). A third child with this disease did not respond to B₁₂ but subsequently responded to folic acid. It is suggested that megaloblastic anemia of infancy may be a syndrome rather than a single entity.

An addendum states that 2 additional patients with megaloblastic anemia have been treated with vitamin B₁₂. One responded to this vitamin the other did not, but experienced a remission when folic acid was given.

*Pediatrics University School of Medicine
Harvard Medical School*

180. LUNBY A. L., and WHEELER, W. E. *Megaloblastic anemia of infancy II—Failure of response to vitamin B₁₂, and the metabolic role of folic acid and vitamin C*, *Health Center J* 3 120, Dec. 1949

Four infants with megaloblastic anemia (which occurs in early infancy is usually corrected with a single course of either liver extracts or folic acid, and does not recur or produce changes in the central nervous system) were treated with vitamin B₁₂. The vitamin was given either parenterally or orally in the form of a natural source (ground beef). Three patients were completely refractory to large doses of vitamin B₁₂ (0.025 to 0.1 mg.) intramuscularly. Their subsequent response to folic acid, refined liver extract, vitamin C, and certain food substances suggest that both pteroylglutamic acid (PGA) and vitamin B₁₂ are necessary for the nucleoprotein metabolism of blood formation and that even large doses of one of these substances are ineffective if the other is not physiologically available. Relatively large doses of one may enhance the effect of small amounts of the other but if the deficiency of one is great enough the other cannot produce an effective hematopoietic response.

The role of vitamin C in megaloblastic anemia of infancy is probably not a direct hematopoietic action but a sparing effect on PGA.

The primary cause of megaloblastic anemia of infancy appears to be, in most cases, a physiologic deficiency of PGA. Factors involved in its development are inadequacies in the diet, infection, and a low vitamin C intake, all of which reduce or keep at a low level the folic acid reserves which are available for blood formation. The authors feel, therefore, that folic acid is the therapeutic agent of choice in the treatment of this disease, and that vitamin B₁₂ should not be the sole therapeutic agent. Growth disturbance in infants with this disease is presumably due to an associated aberration of their protein metabolism.

*Ohio State University College of Medicine, and
Children's Hospital
Columbus, Ohio*

- 181 STURGEON P., and CARPENTER, G. *Megaloblastic anemia of infancy Response to vitamin B₁₂*, *Blood* 5 458-467 May 1950.

It was found that 8 cases of megaloblastic anemia of infancy showed a specific and complete response to a single intramuscular dose of 25 mcg. or less of vitamin B₁₂. Two other cases showed equivocal responses to similar treatment. It is concluded that vitamin B₁₂ is there

peutically effective in some cases of megaloblastic anemia of infancy. It is suggested that vitamin B₁₂ therapy may be made more effective by concomitant therapy with ascorbic acid.

Children's Hospital, and
University of Southern California
Los Angeles, Calif.

182. MAY C. D., NELSON E. N., LOWE, C. V., and SALMON R. J. Pathogenesis of megaloblastic anemia in infancy. An interrelationship between pteroylglutamic acid and ascorbic acid, *Am. J. Dis. Child.* 80 191-206, Aug. 1950.

University of Minnesota Medical School
Minneapolis, Minn.

183. MAY C. D., NELSON E. N., SALMON R. J., LOWE, C. V., LIENKE, R. J., and SUNDBERG R. D. Experimental production of megaloblastic anemia in relation to megaloblastic anemia in infants, *Bull. Univ. Minnesota Hospitals* 21 208, 1950 (cited in *Blood* 5 714 Aug. 1950).

184. WOODRUFF C. W., and PETERSON J. C. The treatment of megaloblastic anemia in infancy. *Post grad. Med.* 10 189-191, Sept. 1951.

Infants that develop megaloblastic anemia have frequently been fed almost exclusively on milk or formulas largely derived from milk. Infections and inadequate intakes of ascorbic acid are often precipitating factors. Folic acid is effective given parenterally in large doses (5 to 15 mg. daily) or orally. The authors have seen adequate response to as little as 0.2 mg. a day orally. Vitamin B₁₂ is effective in many cases but is sometimes completely ineffective in others. For uncomplicated megaloblastic anemia of infancy the authors recommend 5 mg. of folic acid daily by mouth. At least 100 mg. of ascorbic acid should be given daily or its equivalent as orange juice.

Federick's University School of Medicine
Baltimore, Tenn.

185. RUD E.: Case of macrocytic goat's milk anemia successfully treated with vitamin B₁₂. *Ugeskr. f. læger* 115 1301 Sept. 27 1951.

186. BETKE, K., and GANTERT L. Etiology and therapy of goat's milk anemia, *Deutsche med. Wchnschr.* 76 1341 Oct. 26, 1951 (abstr. J.A.B.A. 148 403, Feb. 2, 1952).

The term "goat's milk anemia" should be applied only to anemia in which a megalocytic blood picture and megaloblastic transformation of the bone marrow develop during the feeding of goat's milk. This anemia resembles pernicious anemia. Case histories are given for 2 infants having goat's milk anemia. Treatment with folic acid and vitamin B₁₂ effected recovery. The course of the reticulocytic crisis indicated that the anemia resulted from deficiencies of both vitamin B₁₂ and folic acid.

187. RICKARDS, A. G.: Megaloblastic anaemia of infancy. *Brit. M. J.* 1 1226-1227 June 7 1952.

An 8 month old baby girl with megaloblastic anemia of infancy responded to folic acid and vitamin B₁₂. Five

months later she relapsed, and this time she was treated with folic acid. She is now receiving a maintenance dosage of 10 mg. of folic acid daily.

Other cases of megaloblastic anemia of infancy and juvenile pernicious anemia appearing in the literature are reviewed briefly and the etiology of megaloblastic anemia of infancy is discussed.

Royal Lancaster Infirmary
Lancaster

CHILDHOOD

188. DAVIS R. W., CHRISTIAN R. M., ERVIN D. M., and YOUNG L. E. Pernicious anemia in childhood: report of case in six year old girl responding to refined liver extract, folic acid and vitamin B₁₂ in successive relapses. *Blood* 4 1361 1966, Dec. 1949.

This case presentation of megaloblastic anemia without specific neurologic complications in a 6 year old girl provides an example of childhood pernicious anemia. It is regarded as such despite the fact that a small amount of free hydrochloric acid was present in the gastric juice after injection of histamine. Following administration of refined liver extract, folic acid and vitamin B₁₂ in successive relapses, prompt hematologic responses, which were similar in each case, were obtained. The patient was given 12.5 mcg. of vitamin B₁₂ intramuscularly daily for six days.

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Rochester, N. Y.

189. ROSENZWEIG L., and BRUTON O. C. Pernicious anemia in an eight year old girl: additional observations in a case previously reported as "Nutritional anemia in an infant responding to purified liver extract," *Pediatrics* 6 269-276, Aug. 1950.

Additional studies of a case originally reported by Fouts and Garber (*Am. J. Dis. Child.* 75: 143, 1948) as nutritional anemia in an infant show it to be a case of pernicious anemia in childhood. Up to the age of 8 years the child was given liver extract. This therapy was then discontinued to determine whether the child still required it. After two months, anemia developed. Folic acid treatment, 15 mg. a day by mouth, was given for 24 days, with good results. After about four months the patient was readmitted to the hospital and given vitamin B₁₂, 10 mcg. at weekly intervals. Response was prompt, and this treatment is being continued since the patient prefers it to liver extract, which has sometimes caused mild reactions and gives more pain at the site of injection than does vitamin B₁₂.

Walter Reed General Hospital
Washington, D. C.

190. REISNER, E. H., JR., WOLFF J. A., McKAY R. J., JR., and DOYLE, E. F.: Juvenile pernicious anemia, *Pediatrics* 8 88-106, July 1951.

Macrocytic anemia associated with megaloblastic bone marrow resulting from failure of absorption of vitamin B₁₂ due to lack of the intrinsic factor of Castle present in normal gastric juice, is known as Addisonian

pernicious anemia. This disease, most common in the sixth decade and ordinarily associated with histamine-refractory achlorhydria, has seldom been reported in children; in only 7 cases from the literature can this diagnosis be accepted if the absence of hydrochloric acid after the administration of histamine is specified as a criterion. The authors, however, believe that intrinsic factor deficiency may occur without achlorhydria.

Case histories are presented of 4 children who had what is believed to be true pernicious anemia although achlorhydria was not a factor in 3 of them. All 4 patients responded to liver extract and to vitamin B₁₂ given parenterally. In 3 of whom vitamin B₁₂ was given by mouth no response was obtained. Two of them, however, subsequently responded to oral B₁₂ with normal gastric juice. The other 2 patients developed histamine refractory achlorhydria while in hematologic remission, which is interpreted as proof that the anemia was due to deficient intrinsic factor in the gastric juice. Three of these patients showed evidence of disease of the spinal cord in 2 of them symptoms were severe. The symptoms were compatible with a diagnosis of dorsolateral sclerosis and improved with anti-pernicious anemia therapy. In 1 of these patients, folic acid treatment aggravated the neurologic lesions.

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Belmont Hospital,
Columbia University College of Physicians and Surgeons, and
Belmont Hospital
New York, N. Y.

- 191 PIERCE, N. L.: *Management of anemia in child hood*, Postgrad. Med. 11 68-73, Jan. 1952.

The various anemias of childhood are discussed. Among the treatments suggested are the administration of folic acid in "goat's milk" nutritional anemia, folic acid in megaloblastic anemia in infancy with ascorbic acid if there is a deficiency in this vitamin, ACTH in acquired hemolytic anemia, with repeated transfusions and even splenectomy in chronic cases, and ACTH in some second ary types of aplastic and hypoplastic anemias, particularly those due to toxic agents. In the summary it is stated that vitamin B₁₂ is one of the agents indicated in anemias in children; specific mention of its use is in the section on megaloblastic anemia, in which this vitamin is said to be less effective than folic acid.

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Chicago, Ill.

OLD AGE

192. BASYLEWYCZ, I. V.: *Pernicious anemia in old age*, Rhode Island M. J. 34: 641-644, 664, Dec. 1951.

The author believes that since old people, as a rule, do not respond to liver therapy as promptly as younger patients, the newer treatment of pernicious anemia, with use of vitamin B₁₂, has a great future in the aged, as well as in young people. Concomitant administration of B complex vitamins is especially valuable in elderly patients. Causes of vitamin B₁₂ deficiency in the aged include inadequate diet, insufficient assimilation of available vitamin B₁₂ resulting from difficulties in chewing, gastric achylia and pancreatic disorders, possible inability of the

liver to store vitamin B₁₂, and possible impaired utilization of vitamin B₁₂ by aging tissues.

Rhode Island State Hospital for Mental Diseases
Warrenton, R. I.

OTHER ANEMIAS

- 193 SEBRELL, W. H.: *Anemias caused primarily by malnutrition*, Federation Proc. 8 568-578, June 1949.

The literature is reviewed with special reference to the microcytic or normocytic anemias associated with riboflavin, pyridoxine, pantothenic acid, niacin, amino acid, and vitamin C deficiencies, and with reference to the macrocytic anemias and the role of pteroylglycine and vitamin B₁₂.

- 194 KERPPOLA, W.: *Observations on the phosphatase content of blood and bone marrow cells in normal and pathologic hemopoiesis*, Blood 11 454-465, May 1951.

In this article reference is made to a case of "achrestic" anemia in which the patient is described as having responded weakly to treatment with vitamin B₁₂.

University of Helsinki
Helsinki, Finland

195. DASHER, W. A., and BOGEN E.: *Antianemic factors in the treatment of iron deficiency anemias*, Ann. West. Med. & Surg. 6 85-87 Feb. 1952.

The addition of the so-called antianemic factors (folic acid, vitamin B₁₂, powdered stomach and liver fraction) to iron in the treatment of patients with iron deficiency anemia did not result in greater improvement. In fact the patients on iron alone did better than the others. Indiscriminate use of antianemic factors is wasteful and contraindicated in treatment of iron deficiency anemias.

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Olathe, Kans., Calif. (E. B.)

196. SILVER, H. K.: *Mediterranean anemia*, California Med. 76 162-164, March 1952.

History, incidence and distinguishing features of the disease are discussed. In its severe form, early and periodic transfusions of whole blood offer the only effective treatment of this anemia. Numerous other types of treatment, including splenectomy, B₁₂, liver iron, and other hematinics have not been of value, in the author's experience. He states that in the mild form of this anemia, specific treatment is not necessary.

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- 197 HUNTER, O. B., Jr.: *L. citrovorum factor in refractory anemia*, Ann. West. Med. & Surg. 6 275-278, May 1952.

Six cases of anemia refractory to the usual therapeutic measures are reported. Citrovorum factor produced a slight increase in the reticulocyte response over that resulting from the use of folic acid or vitamin B₁₂ the effect was not maintained after therapy was discontinued, and a second course was not effective.

case. In one patient, a blood count considerably better than previously noted was maintained for six months by citrovorum factor. There is no indication, however, that citrovorum factor will maintain the red cell count on an adequate level.

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Washington, D. C.*

198. KJERULF-JENSEN K., and SCHWARTZ, M.
Coat, leukemia, and polycythemia, Lancet 1 599
March 21, 1953 (in Letters to the Editor)

A patient was treated with 80 mcg. of vitamin B₁₂ and 2 Gm. of hog's stomach extract daily for anemia accompanying gout. After two and one-half months polycythemia developed, its peak occurring two weeks after this treatment was discontinued. The blood count showed red cells 7,600,000 per cu. mm., Hb 121 per cent, leukocytes 26,000 per cu. mm., thrombocytes 516,000 per cu. mm. Stereal marrow biopsy showed intense hyperplasia with a pronounced shift to the left and no megaloblasts suggesting polycythemia. Moderate splenomegaly had developed. No treatment was given for three months, then vitamin B₁₂ and hog's stomach extract was given for one month. The bone marrow showed the same picture as before, but the polycythemia did not recur.

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Copenhagen, Denmark*

ANEMIAS IN GENERAL

199. EDITORIAL. *Vitamin B₁₂ in anemia*, Am. Prof. Pharm. 14 427 May 1948.

200. SPIES, T. D., STONE, R. E., and ARAMBURU T.: *Observations on the antianemic properties of vitamin B₁₂*, South. M. J. 41: 522-523, June 1948.

Two patients with pernicious anemia, 2 with nutritional macrocytic anemia, and 1 with nontropical sprue were given crystalline vitamin B₁₂. Each case had been under observation for years. Four of the patients received a single injection of 6 mcg. 1 patient with pernicious anemia was given 15 mcg. in one dose. Within three to five days all the patients showed striking clinical improvement, which included an increase in their feeling of well being, mental alertness, strength and vigor and complete relief of soreness and burning of the tongue and mouth. There was a rise in reticulocytes in each case, reaching a peak from the fifth to the ninth day. This was followed by an increase in red blood cells and hemoglobin and, with the exception of 1 patient with nutritional macrocytic anemia, in white blood cells also. The response in the 2 cases of nutritional macrocytic anemia and in the 1 case of nontropical sprue shows that vitamin B₁₂ has positive hemopoietic activity in these diseases as well as in pernicious anemia.

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201. SPIES, T. D., STONE, R. E., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and ARAMBURU T.: *Thymine, folic acid, and vitamin B₁₂ in nutritional macrocytic anemia, tropical sprue and pernicious anemia*, Lancet 2 519-522, Oct. 2, 1948.

The hematologic responses of the same individual to thymine, folic acid, and vitamin B₁₂ were compared in 3 patients with macrocytic anemia in relapse. One patient had nutritional macrocytic anemia, one tropical sprue, and one pernicious anemia. Each patient was admitted to the hospital three times for treatment of anemia in about the same degree of relapse. During the test periods the patients were given a diet free from meat and meat products. Thymine and folic acid were taken orally over periods of 10 or 14 days. Vitamin B₁₂ was administered in a single intramuscular injection. Satisfactory hematologic response and definite clinical improvement were obtained with each of the three substances in each case—in the patient with nutritional macrocytic anemia with 6 Gm. of thymine or 10 mg. of folic acid daily or with 6 mcg. of vitamin B₁₂; in the patient with tropical sprue with 10 Gm. of thymine or 10 mg. of folic acid daily or with 23 mcg. of vitamin B₁₂; and in the patient with pernicious anemia with 6 Gm. of thymine or 10 mg. of folic acid daily or with 15 mcg. of vitamin B₁₂. Approximately equal reticulocyte peaks were obtained with folic acid and vitamin B₁₂ within similar intervals (about the seventh day in 2 patients and the eleventh day in the third). Except in the patient with pernicious anemia, the reticulocyte response was less pronounced under thymine. With all three substances there was progressive increase in the red blood cell count and hemoglobin level throughout the period of study.

The patient with nutritional macrocytic anemia had a more severe glossitis at the time of his third relapse than on the two previous occasions. This disappeared in five days following treatment with vitamin B₁₂ and the patient gained strength quickly and steadily. The patient with pernicious anemia showed only slight clinical improvement while on thymine and his stomatitis and glossitis remained unchanged. Folic acid had a more favorable effect on these symptoms but they did not disappear entirely. Glossitis, stomatitis, and weakness were severe in this patient at the time of his third relapse. Following treatment with vitamin B₁₂, the hyperemia of the tongue and oral mucosa began to fade after 48 hours, and the mucosa appeared almost normal five days after the injection; the papillae had regenerated by the thirteenth day. In this case the blood response to vitamin B₁₂ was paralleled by remarkable improvement in strength and vigor.

From these and their other studies, the authors conclude that, while thymine, folic acid, and vitamin B₁₂ are each effective in the above three syndromes, to produce comparable responses the required dose of thymine is several thousand times that of folic acid, and the required dose of folic acid several thousand times the effective dose of vitamin B₁₂. This suggests that vitamin B₁₂ is by far the most potent antianemic substance known to date.

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202. BETHELL, F. H., MEYERS M. C., and NELIGH, R. B.: *Vitamin B₁₂ in pernicious anemia and peripheral macrocytic anemia*, J. Lab. & Clin. Med. 33: 1477-1478, Nov. 1948 (in Soc. Proc.) (also in Proc. Central Soc. Clin. Research 21 27-28, 1948)

Four patients with pernicious anemia in relapse were treated with vitamin B₁₂ in daily intramuscular injections of 1 microgram until there was no further reticulocyte response, after which injections were given at varying intervals up to 25 days, the dosage being adjusted to provide the equivalent of 1 mcg. a day. Treatment was continued for 10 to 20 weeks. Three of the patients had a greater hematologic response than would have been expected from daily injections of 1 U.S.P. unit of liver extract. The fourth patient, who was given 1 mg. of the folic acid antagonist, aminopterin, daily for 2 days before and for the first 14 days during treatment with vitamin B₁₂, had a delayed and suboptimal reticulocyte response, but after aminopterin was discontinued the red cell count rose rapidly. All 4 patients had normal hematologic values after six to eight weeks. Paresthesias were relieved and ataxia decreased in 3 patients with nervous system involvement, and glossitis, which was troublesome in 3 patients, disappeared promptly under vitamin B₁₂ treatment. One case of macrocytic anemia following pregnancy failed to respond to daily doses of 1 mcg. vitamin B₁₂ for 10 days but later responded to 10 mcg. of folic acid daily by mouth. Assay of the feces of 4 patients with untreated pernicious anemia revealed stimulation for the growth of *Lactobacillus lactis* equivalent to 0.3 to 1.8 mcg. vitamin B₁₂/Gm. of dried feces. This indicates a daily fecal output of vitamin B₁₂ by patients with pernicious anemia many times greater than that necessary to produce remission when introduced parenterally. This suggests that in pernicious anemia the absorption of vitamin B₁₂ derived either from dietary sources or by intestinal bacterial synthesis may be defective.

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203. RAVINA, A. *Un progrès décisif de thérapeutique anti-anémique la vitamine B₁₂ (Conclusive progress in anemic therapy vitamin B₁₂)* Presse méd. 57: 89-90, Jan. 22, 1949

American and English articles regarding vitamin B₁₂ are reviewed.

204. SPIES, T. D., SUAREZ, R. M., GARCIA LOPEZ, G., MILANES, F., STONE, R. E., LOPEZ TOCA, R., ARAMBURU T., and KARTUS S. *Tentative appraisal of vitamin B₁₂ as a therapeutic agent*, J.A.M.A. 139: 521-525, Feb. 10 1949

Vitamin B₁₂ was used in the treatment of 21 patients with macrocytic hyperchromic anemia. Of these, 4 had nutritional macrocytic anemia, 1 had nontropical sprue, 11 had tropical sprue, and 5 had pernicious anemia. The vitamin was given intramuscularly and the total dose ranged from 4 to 25 mcg. The administration of vitamin B₁₂ was followed by excellent clinical and hematologic improvement in all 21 patients. They felt stronger and their appetites returned four or five days after the vitamin was injected. The patients with sprue noted a decrease in abdominal distention and in the volume of the stools after six or seven days.

Vitamin B₁₂ brought about improvement in 14 patients with pernicious anemia. Nine of these had severe and 5 had mild subacute combined degeneration. Within two weeks after the administration of vitamin B₁₂ the

peripheral nerve and posterior column involvement was greatly improved in these patients.

Several additional patients also were treated with vitamin B₁₂. One had both pernicious anemia and sprue, and the symptoms of both diseases subsided after oral treatment with 25 mcg. vitamin B₁₂ which had been incubated with gastric juice obtained from a patient with duodenal ulcer. This patient had previously failed to respond to treatment with 50 mcg. vitamin B₁₂ concentrate orally. Apparently the human gastric juice is able to effect the utilization of vitamin B₁₂.

205. SPIES T. D., STONE, R. E., GARCIA LOPEZ, G., LOPEZ TOCA, R., and ARAMBURU T. *Observations on vitamin B₁₂ as an anemic substance* Internat. Zschr. f. Vitaminforsch. 21: 328-340 1949

206. STURGIS C. C. *Advances in our knowledge concerning the etiology and treatment of hematological disorders*, Bull. New York Acad. Med. 23: 84-99 Feb. 1949

In anemia due to chronic infection, penicillin, streptomycin, or the sulfonamide drugs should be employed either alone or in combination with surgical measures. Folic acid may be of value in the treatment of sprue, the macrocytic anemias of pregnancy, nutritional macrocytic anemia, and anemia due to intestinal disturbances, but it is without beneficial effect on the neurologic lesions of pernicious anemia. Improvements in blood picture and neurologic manifestations have been induced with vitamin B₁₂. It may be possible to substitute vitamin B₁₂ for liver extract therapy in patients who develop allergic reactions to the latter.

Also discussed are treatment recommendations in agranulocytosis, Hodgkin's disease, chronic myelogenous and lymphatic leukemias.

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207. STURGIS C. C. *The treatment of hematologic disorders*, Postgrad. Med. 5: 300-306, April 1949

This is a general article on the treatment of hematologic diseases, including pernicious anemia, Hodgkin's disease, leukemia, and polycythemia.

The author has treated 6 cases of pernicious anemia with vitamin B₁₂. The dose usually used was 1 microgram per day but 2 of the patients received only 0.45 mcg. per day and showed a definite, though substandard response.

208. SPIES, T. D., GARCIA LOPEZ, G., MILANES F., LOPEZ TOCA, R., and ARAMBURU T. *A note on the oral versus parenteral administration of vitamin B₁₂*, South. M. J. 42: 528-531, June 1949

The effect of vitamin B₁₂ administered orally was studied in 4 patients with pernicious anemia, 4 with tropical sprue, and 4 with nutritional macrocytic anemia. All 12 patients were in relapse, and all had macrocytic hyperchromic anemia, a red blood cell count of 2,600,000 or less, a color index of 1.0 or more, and megaloblasts of the sternal bone marrow. These patients

hospitalized and kept on a diet devoid of meat and meat products until baseline determinations of blood cell counts, hemoglobin, and reticulocyte counts had been made. Vitamin B₁₂ was then administered orally. After four or five days there was general clinical improvement, reticulocytosis reached a peak around the seventh day and subsequently there was an increase in red blood cells and hemoglobin. The megaloblastic arrest of the bone marrow tended to disappear and the marrow became more normal in appearance. Because of scarcity of material the response obtained was somewhat submaximal. In general, thirty to fifty times as much vitamin B₁₂ is required when given orally as when given by injection. If the vitamin is incubated with normal human gastric juice, however, only five to ten times the parenteral dose is required. Responses were obtained from 10 or 30 mcg. intramuscularly or 800 mcg. orally.

In 2 of the patients with pernicious anemia, combined system disease developed despite treatment with 80 mcg. vitamin B₁₂ by mouth. When 30 mcg. was incubated with human gastric juice and administered orally to these patients, the blood responded and the acute signs of combined system disease disappeared. Parenteral administration has the advantages of requiring less material and of permitting more accurate supervision.

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- 209 SPIES, T. C., GARCIA LOPEZ, G., MILLANES, F., STONE, R. E., LOPEZ TOCA, R., ARAMBURU T., and KARTUS, S.: *Observations on the effect of an animal protein factor concentrate on persons with the macrocytic anemia of pernicious anemia, of nutritional macrocytic anemia and of sprue and on persons with nutritional glossitis* Blood 4 819-826, July 1949.

Animal protein factor concentrate produced a hematologic response in each of 5 cases of pernicious anemia in relapse, 4 cases of nutritional macrocytic anemia in relapse, and 3 cases of tropical sprue in relapse. It also caused the redness and soreness of the tongue to disappear in 3 cases of nutritional glossitis not associated with anemia. The concentrate, a product of microorganisms, was administered by intramuscular injection in dosages ranging from a total of 5 cc. over a period of 23 days to 5 cc. daily for 14 days. Representative cases are described briefly. In the case illustrating the response of pernicious anemia to animal protein factor there was no objective evidence that neurologic symptoms were improved although there was general symptomatic improvement. Treatment had to be discontinued in the case of glossitis described because of local hyperemallicity to the concentrate.

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210. JONES, E., DARBY W. J., and TOTTER, J. R.: *Pernicious anemia and related anemias treated with vitamin B₁₂* Blood 4 827-844, July 1949.
- Study of the hematologic data on 8 patients with anemia who were treated with vitamin B₁₂ for

periods up to six months indicates that a total dosage averaging less than 0.75 mcg. daily when the vitamin is given parenterally and at intervals of several days, will not establish and maintain as high blood levels as will adequate liver therapy. All of the patients responded hematologically to vitamin B₁₂ and 2 who had mild neurologic symptoms improved. Parenteral daily doses of 1.0 mcg. promoted good erythropoiesis in one patient, but erythropoiesis seemed to be more consistently rapid in patients who received 2.5 mcg. or more daily. It would appear that the average initial daily dose should be about 3.0 mcg. In order to obtain a maximum rate of hemopoiesis. The observations in these cases again emphasized the unreliability of the reticulocyte count as a quantitative measure of the activity of an anti-pernicious anemia agent or of the adequacy of therapy in a given patient. Vitamin B₁₂ caused a decrease in fecal urobilinogen, an effect that has been observed by other investigators using liver extract for pernicious anemia and by the authors following administration of folic acid for this disease. No effect of vitamin B₁₂ on urinary pteroylglutamic acid (PGA) or porphyrin excretion could be detected.

The hematologic response of a patient with nutritional macrocytic anemia to vitamin B₁₂ was equal to the responses that had previously been obtained in this patient with liver extract, and there was concomitant symptomatic improvement. A patient with sprue responded to the vitamin hematologically but after receiving large amounts over a period of 27 weeks his erythrocyte count had not equalled that previously reached with liver extract or folic acid. A hematologic relapse had occurred, however while the patient was receiving presumably adequate amounts of folic acid. This fact and the incomplete response to vitamin B₁₂ suggest that hemopoietic factors in addition to folic acid and B₁₂ may be required by some patients for a maximum erythrocytic response. The absorptive defect in a patient with anemia associated with intestinal lipodystrophy was not altered by administration of vitamin B₁₂.

- 211 DARBY W. J., and JONES, E.: *Observations on two newer hemopoietic vitamins—vitamin B₁₂ and animal protein factor* Am. J. Med. 7 241 Aug 1949 (In Soc. Proc.)

The responses to crystalline vitamin B₁₂ administered parenterally to 11 patients with pernicious anemia, nutritional macrocytic anemia, or sprue indicate the approximate equivalence of 1 microgram of vitamin B₁₂ to 1 U.S.P. unit of anti-pernicious anemia liver extract. In pernicious anemia, the remissions produced by B₁₂ have been characterized by relief of glossitis, an increased sense of well-being, an initial hemopoietic response, maturation of the megaloblastic marrow weight gain, decrease in fecal urobilinogen and, in 2 patients, disappearance of early neurologic symptoms. In 2 cases of sprue, the results have been less clearly defined, and a greater quantitative requirement for B₁₂ in this syndrome is probable. Nutritional macrocytic anemia has responded in a manner comparable to pernicious anemia. Evidence of approximate correspondence of the activity of the animal protein factor in patients with pernicious anemia (parenteral administration) and in the chick, has been found. Since these two factors are effective parenterally it does not appear that they correspond to Castle's extrin-

sic factor although their association with animal protein would imply that they may

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212. SPIES T. D., STONE, R. E., GARCIA LOPEZ, G., MILANES, F., and LOPEZ TOCA, R.. *Vitamin B₁₂ by mouth in pernicious and nutritional macrocytic anaemia and sprue*, Lancet 2 454-456, Sept. 10, 1949

Illustrative cases are summarized to indicate the effect of oral vitamin B₁₂ therapy in pernicious anemia, nutritional macrocytic anemia, and tropical and nontropical sprue. The results reported show that such treatment if adequate is often effective. Most patients, however respond slowly to oral B₁₂ even when given in 30 to 60 times the required parenteral dose. The authors conclude that vitamin B₁₂ should always be administered parenterally if there is severe cardiac failure, severe neural degeneration, or severe alimentary disturbance, and at least initially in severe cases of megaloblastic anemia. Although an allergic reaction to parenteral vitamin B₁₂ has not yet been encountered, this is considered a possibility in which case oral treatment may be useful. The minimum, maximum, and optimum doses of vitamin B₁₂ vary with the patient and also from time to time in the same patient, and therefore no set routine of treatment can be followed satisfactorily.

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213. BOEHRER, J. J.: *Recent advances in pernicious anemia, hypochromic anemias and hemolytic anemias*, Minnesota Medicine 32 889- Sept. 1949
214. CHEYMOL, J.: *Vitamin B₁₂ and antipernicious anemia factor* Semaine d. hôp. Paris 23 3305-Oct. 30, 1949
215. STURGIS, C. C. *Recent advances in treatment of hematologic disorders* J.A.M.A. 141: 969-973, Dec. 3, 1949.

This review article states that liver extract given intramuscularly is the treatment of choice for pernicious anemia, but vitamin B₁₂ may prove to be equally effective when administered intramuscularly or perhaps orally with intestinal extract. Folic acid intramuscularly or orally is highly effective for all varieties of macrocytic anemia except pernicious anemia, and is the most satisfactory treatment for the macrocytic anemia of pregnancy in which liver extract and vitamin B₁₂ are often, if not always, ineffective.

Therapy in myelogenous leukemia, Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma is also discussed.

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216. SMITH, E. L.: *Folic acid, vitamin B₁₂ and anaemia*, Nature 164 986-987 Dec. 10, 1949

This is a review of a symposium on folic acid, vitamin B₁₂ and anemia. The papers read present a survey of the historical development of liver and related therapy of anemia, and of the work on folic acid and vitamin B₁₂ and summarize present day's chemical, biochemical and clinical results.

217. UNGLEY C. C. *Recent work on vitamin B₁₂*, Lancet 1 353-354, Feb. 23, 1950 (In Soc. Proc.)

The action of vitamin B₁₂ and some kindred substances isolated by Smith was studied in two groups of cases: idiopathic Addisonian anemia with gastric atrophy and permanent loss of Castle's intrinsic factor which leads to deficient absorption of B₁₂, and non-Addisonian megaloblastic anemias such as those associated with pregnancy and intestinal disorders.

When an initial dose of 11 to 80 mcg. was given, the increase of red blood cells on the fifteenth day was proportional to the logarithm of the dose. Up to the fifth day there was little effect with any of these single doses, and after the fifteenth to twentieth day the red cell curve flattened. It was found that for maintenance, in the absence of subacute combined degeneration of the cord, 10 mcg. every two weeks was sufficient over periods up to 18 months.

Of 8 patients receiving B₁₂ during periods of 9 to 19 months, 4 showed improvement greater than that expected with liver extract, 2 showed improvement as great, and 2 showed less. The author concluded that B₁₂ was as effective as liver extract in Addisonian anemia as well as in subacute combined degeneration of the cord. Weekly doses of 40 mcg. were usually sufficient in the first six months, with 80 mcg. weekly for maintenance.

Twenty patients with Addisonian pernicious anemia received Smith's "unnamed crystalline substance," obtained from *Streptomyces griseus*. The initial doses were as effective as B₁₂ with regard to blood picture, glossitis and neurologic symptoms. One patient with apparent Addisonian anemia, who had been treated six months previously for syphilis, showed poor response to B₁₂. 2.5 mg. of folic acid daily (total, 15 mg.) caused a satisfactory reticulocyte rise. A subsequent 10 mcg. of B₁₂ produced a renewed rise in the red cell level which had begun to fall.

Oral administration of B₁₂ (or of the "unnamed substance") gave poor results: a single 5 mcg. injection was more effective than 1,920 mcg. given orally over 24 days. However when 50 cc. of normal unfiltered gastric juice was added to a 5 mcg. daily oral dose of B₁₂ a satisfactory response was obtained, but it still required 50 mcg. of B₁₂ orally to equal the effect of 10 mcg. of B₁₂ by parenteral administration. When the gastric juice was passed through Seltz filters, the intrinsic factor activity was removed.

In 2 of 3 cases of megaloblastic anemia with intestinal disorders, much larger (4 to 14 times) doses of B₁₂ were necessary to obtain response than in cases of Addisonian anemia. One of the 3, who did not respond to B₁₂, showed favorable results on receiving folic acid.

Six patients with megaloblastic anemia of pregnancy which were refractory to 65 to 80 mcg. doses of B₁₂ showed excellent response to 2.5 mg. of folic acid.

In referring to the problems still to be solved the author noted that yeast given orally could cause a remission in pernicious anemia. If gastric juice was given adjunctively one-tenth of the dose sufficed this dose was also sufficient in nonaddisonian anemias. Yeast extracts apparently contain no B_{12} yet they seemed to be a source of the extrinsic factor

218. SPIES T. D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and REBOREDO A.: *Anti anemia properties of a reaction product of vitamin B_{12} and the intrinsic factor* South. M. J. 43: 206-208, March 1950.

The authors' experiences with capsules of Berubosyn suggest that the time when macrocytic anemia can be treated with a single capsule per day is near at hand. Each of these capsules contains a reaction product of 9 mcg. of vitamin B_{12} and the intrinsic factor present in 0.33 Gm. of concentrate of hog duodenum. Four patients with macrocytic anemia in relapse (1 with pernicious anemia, 2 with tropical sprue, and 1 with nutritional macrocytic anemia) were treated for 10 days, and each showed some clinical and hematologic improvement. The reticulocytes reached a peak nine to 11 days after treatment was begun. None of the cases gave a maximum response, which indicates that a larger dose is required if treatment is continued no longer than 10 days, but supplies were not available for this purpose.

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219. CAMERON D. G. *Therapy of the blood diseases*, Mod. Med. 18: 61-64, 142-156, April 15, 1950.

This review contains references to liver extract in the treatment of pernicious anemia, and to the use of vitamin B_{12} for the same purpose. Given orally vitamin B_{12} acts like the "extrinsic" factor and given parenterally it acts like the "anti-pernicious anemia" factor. The oral administration of 5 mcg. of vitamin B_{12} daily has no effect on the blood in pernicious anemia when given alone, but produces a maximal response when given with 50 to 150 cc. of normal gastric juice. This vitamin appears to relieve and to prevent subacute combined degeneration of the spinal cord, and it may eventually replace liver extracts in the treatment of pernicious anemia, although liver extract must be considered the treatment of choice until the value of vitamin B_{12} is finally established.

Folic acid relieves all types of macrocytic anemia associated with megaloblastic bone marrow but it does not relieve or prevent neurologic complications in pernicious anemia and may even precipitate them. It can be given parenterally or orally. It is the treatment of choice in pernicious anemia of pregnancy in which liver extract and vitamin B_{12} are often ineffective.

Since leukemia cells contain folic acid which may be necessary for their growth, folic acid antagonists such as aminopterin may produce short remissions in leukemia. The drug is given intramuscularly or orally in daily doses of 1 mg. until toxic symptoms appear and is then continued in smaller amounts.

Urethane has also been used in leukemia, and is effective in the chronic myeloid type.

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220. GOLDSMITH, G.: *Vitamin B_{12}* , Lancet 2: 407 Sept. 23, 1950 (in Soc. Proc.)

"Dr. Grace Goldsmith (U.S.A.) thought that 4 μ g. (of vitamin B_{12}) injected daily gave results poorer than liver extract and reported failure with oral treatment, though in sprue and nutritional macrocytic anemia 5-30 μ g. by mouth daily did evoke some response. She recalled that a good haemopoietic response has been obtained in pernicious anaemia when 5 μ g. of B_{12} is given orally together with a duodenal extract but she did not mention what was the effect of the duodenal extract alone. Finally she suggested that vitamin B_{12} may not maintain pernicious anaemia patients at proper blood levels for long periods."

221. OWREN P. A.: *Vitamin B_{12}* , Lancet 2: 407 Sept. 23, 1950 (in Soc. Proc.)

"In the discussion Prof. P. A. Owren (Norway) produced more evidence that vitamin B_{12} is not the anti-anemic principle and that responses to it are partial. He showed that the prothrombin concentration is low in untreated pernicious anemia and remains low when B_{12} treatment has raised the haemoglobin; folic acid has the same effect, but some liver extracts will restore the picture. He also pointed out that abnormal haemoglobin of the foetal type remains, and macrocytosis persists, with B_{12} treatment."

222. SPIES, T. D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and REBOREDO A.: *Folic acid and vitamin B_{12} in anemia*, GP 2: 37-43, Sept. 1950.

An intensive two-year study of selected cases of anemia indicates that folic acid and vitamin B_{12} should be used together in practical everyday therapy. One case history is given which is representative of 8 patients with macrocytic or pernicious anemia of pregnancy who responded to folic acid. In a second illness, the patient failed to respond to vitamin B_{12} until folic acid was added, whereupon rapid improvement began. She continued well under maintenance treatment with folic acid.

A second case report is representative of 8 patients who responded hemopoietically and clinically to folic acid but required vitamin B_{12} to relieve neurologic symptoms. Maintenance treatment consisted of an injection of 30 mcg. vitamin B_{12} twice a month. A third case is illustrative of 10 elderly patients with pernicious anemia who had been treated for 20 years or more. Their blood levels remained low during treatment with 150 to 250 mcg. vitamin B_{12} a week but became normal when folic acid was added. Maintenance therapy with 60 mcg. of vitamin B_{12} a week and 10 mg. of folic acid daily was effective. Since both folic acid and vitamin B_{12} have specific functions as well as functions in common, the giving of both for complete and continued hemopoiesis in properly selected patients is recommended.

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223. WOODS, D. D.: *Folic acid vitamin B₁₂ and anemia*, J Pharm. Pharmacol. 2: 537-540, Sept. 1950.

A review; 27 references.

224. BARNARD R. D.: *Splenectomy for blood dyscrasias* Mod. Med. 18: 16, Oct. 1, 1950 (In Correspondence)

Oral doses of Streptomyces-derived antibiotics (streptomycin, chloromycetin, streptomycin, and some not yet characterized) in quantities just sufficient to suppress the ordinary proteolytic intestinal flora and to substitute a predominantly saccharolytic gram-positive flora have been observed to cause such rapid clinical and hematologic improvement in 8 successive patients with hemolytic anemia that the writer thinks splenectomy for this disease may soon be discarded.

Work in progress indicates that peptones, proteoses, arising from proteolytic activity of the flora in an intestinal tract of heightened permeability may be responsible for many hemopoietic arrests. Circulating peptones may be curtailed by intestinal coliform organism suppression (by oral antibiotics) by altering intestinal permeability and the pathway of protein catabolism (cor. biotherapy) or by bolstering cholinesterase synthesis (B₁₂ and folic acid)

These effects may overlap, that is, oral antibiotics or folic acid may cause salt and water retention indistinguishable from that of corticotherapy. folic acid and B₁₂ have been found to have some coliform-suppressor antibiotic activity

Terramycin has produced two complete remissions in infantile megaloblastic anemia. The daily dose was 150 mg.

Radiation anemia and that of glomerulonephritis both appear to be more responsive to minimal oral antibiotic therapy than to any other agency not excluding transfusion.

These results would seem to warrant a trial of oral antibiotic therapy in refractory anemias before resorting to splenectomy in cases which are not urgent.

L. L. H. Y.

225. APPEL, W.: *Rationale of therapy of blood diseases*, J Michigan State M. Soc. 49: 1183-1190, 1204 Oct. 1950.

The role of vitamin K in clotting is discussed in an outline of the clotting mechanism. Therapeutic administration of large quantities of vitamin K is indicated when excessive amounts of dicumarol have been given to a patient. Vitamin B₁₂ is mentioned as containing the erythrocyte maturing factor as does folic acid, but B₁₂ is apparently able to prevent and control nervous system involvements. Folic acid antagonists seem to be of most value in acute leukemia. HN₂ does not produce satisfactory remissions. HN₂ in chronic myelogenous leukemia produces temporary palliative results by damaging susceptible cells. Use of too large doses of HN₂ in chronic lymphatic leukemia may damage hemopoietic tissue.

Kahnman, N. H.

226. SPIES T. D., GARCIA LOPEZ, G., MILANES F., LOPEZ TOCA, R., REBOREDO A., and STONE, R. E.: *The response of patients with pernicious anemia, with nutritional macrocytic anemia and with tropical sprue to folic acid or citrovorum factor* South. M. J. 43: 1076-1082, Dec. 1950.

227. WESTERMAN D. D.: *Folic acid and vitamin B₁₂ as anti-anemia factors* J Home Econ. 42: 199-203, 1950.

A brief review of the literature on investigations leading up to the discovery and isolation of folic acid and vitamin B₁₂.

228. BARNARD R. D., CARACAPPA, J. M., SCHWARTZ, M., GORDON G. B., and MOLD-ORIEL, A.: *Further observations on the response of erythroblastic, megaloblastic, and radiation anemias to Streptomyces-derived "animal protein factor" and antibiotics*, New York State J. Med. 51: 1739-1745 July 15 1951

Previously reported work (Barnard and Fox: Ohio State M. J. 46: 784, 1950 [Abstr. 325]) has shown that a crude Streptomyces griseus residue concentrate, marketed as "animal protein factor" for fortification of animal feed, has an arresting or reversing action on anemia in patients with acute leukemia. This action appears to be due to some hematopoietic factor other than B₁₂, since it is not produced by more refined preparations of this vitamin. Preparations from species of Streptomyces which do not produce antibiotics did not give comparable results, which indicates that the hematopoietic effect may be due to the antibiotic content of the effective preparations.

Streptomyces griseus concentrate is a by-product of streptomycin manufacture which, unlike streptomycin but like terramycin, streptomycin and chloramphenicol, is effective by oral administration. It produces systemic effects in lower doses than do these antibiotics, and these effects are not attributed to bacteriostatic action although the bacteriostatic action of minute oral doses of antibiotics is also present, acting by changing the fecal bacteria from a predominantly gram-negative proteolytic to a gram-positive saccharolytic type.

Seven case histories are presented and summarized as follows: "A crude Streptomyces griseus residue was found to have marked hematopoietic effects similar to those exhibited by adrenocorticoids in hemopoietic suppressive conditions such as erythroblastic arrest (acquired hemolytic) anemia, megaloblastic anemia, and radiation myelopathy. This effect could not be attributed to the B₁₂ content of the residue, and two opposed cases in each category are presented to show that the same effect can be produced by a coliform-aerogenes suppressor antibiotic. It is postulated that ingestion of such an antibiotic, both by direct depressant effect on intestinal proteolytic organisms and by the indirect implantation and maintenance of a saccharolytic flora, may limit the absorption of cholinergic proteoses. An enhanced production of erythrocyte cholinesterase is thereby permitted, and the latter may instigate accelerated hemopoiesis. At time, the possibility that hemopoiesis may be

substances derived from the non-sporulating gram-positive intestinal organisms which flourish during this therapy is not ruled out. Whatever the mechanism, the hematologic effect of sustained, small, oral antibiotic dosing holds promise of wide clinical application, not only to the management of the myelosuppressive hematologic dyscrasias, but to all allergic, atopic and adaptive diseases as well.

"Treatment failures with this modality seem to have occurred predominantly where intestinal coliform suppression did not take place or where intolerance to the implanted saccharolytic flora developed. In some instances, profound adrenocorticotrophic effects, among which salt and water retention were pronounced, forced an abandonment of the antibiotic dosage schedule."

A footnote states that sodium and water retention due to protracted therapy with terramycin or the Streptomycin animal protein factor must now be emphasized because about one of seven patients on such therapy develops gross edema which responds to theophyllin-aminocaproic chloride diuresis. It is believed that 5 leu keto patients with diminished cardiac reserve died from congestive failure before the corticoid features of this type of therapy were recognized. In two other instances, symptoms resembling those of Cushing's syndrome appeared.

Lauritzen, N. Y. (R.B.B.); Elwood, R. T. (M.C.); Bromberg, N. Y. (M.C.); and Bromberg, N. Y. (M.C. and J.M.)

- 229 LIMARZI, L. R. *Treatment of blood dyscrasias*, Postgrad. Med. 10 51-56, July 1951

Among the subjects discussed in this review is the use of vitamin B₁₂ in pernicious anemia and some other macrocytic anemias. Megaloblastic anemia of pregnancy is said to respond well to folic acid and liver extract, but not to vitamin B₁₂. The best treatment for megaloblastic anemia of infancy and childhood may be 200 mg. of ascorbic acid, 15 mg. of folic acid, and injectable crude liver extract given intramuscularly at first and then orally.

Included in the article are discussions of therapy in Hodgkin's disease, chronic myeloid leukemia, multiple myeloma, polycythemia vera, and agranulocytosis.

230. EDITORIAL: *Vitamins and blood regeneration*, J.A.M.A. 147 979-981, Nov 3, 1951

In this editorial, pertinent literature on the effects of vitamins, particularly vitamin B₁₂ and folic acid in various anemias, is discussed, 13 references being cited.

- 231 GIRDWOOD R. H.: *Reports of societies vitamin B₁₂ therapeutic value*, Brit. M. J. 1 161, Jan. 19 1952.

"Dr. R. H. Girdwood dealt with the complicated interrelationships between vitamin B₁₂, folic acid, and folic acid both in the nutrition of micro-organisms and in the cure of anemia. He concluded that both vitamin B₁₂ and folic acid were necessary for normoblastic blood, and that folic acid was probably converted in the blood to folic acid. Other factors, such as ascorbic acid body to folic acid. Other factors, such as ascorbic acid, and the anti-anemic factor of Wills, were also important, and the picture might be further complicated by the presence of inhibitory factors."

232. UNGLEY C. C. *Reports of societies vitamin B₁₂ therapeutic value*, Brit. M. J. 1 161 Jan. 19 1952.

"Dr. C. C. Ungley discussed the value of vitamin B₁₂ in the treatment of various forms of anemia, and explained that it was only effective when the bone marrow was megaloblastic. Not all forms of megaloblastic anemia, however, responded to therapy. Thus the anemias of pregnancy and the puerperium, as observed in temperate climates, failed to respond to vitamin B₁₂, but were corrected by folic acid. The so-called 'tropical sprue' sometimes responded to vitamin B₁₂. Anemia associated with cirrhosis of the liver was seldom megaloblastic, but, when it was, vitamin B₁₂ might be effective. Anemia after total gastrectomy had been corrected by folic acid, but not by vitamin B₁₂. Anemia produced by a fish tapeworm had been cured either by vitamin B₁₂ or by the expulsion of the worm. The parasites had been found to be rich in vitamin B₁₂, and presumably caused anemia by absorbing supplies otherwise available to its host."

"Dr. Ungley emphasized that, while vitamin B₁₂ could correct not only the blood picture in pernicious anemia but also the glossitis and nervous lesions, folic acid could only temporarily correct the blood picture. Doses of 1 µg. often produced definite improvement, but when patients had nervous lesions or suffered from later current diseases 100 µg. monthly should be allowed."

233. PATEL, J. C. *Necessity of B-complex factors in the treatment of anemias* J Indian Med. Assn. 21 191 194 Feb. 1952 (abstr. Am. J. Digest Dis. 19 235, July 1952)

"In the various anemias seen in India, Patel has found it best to rely upon liver extract, folic acid or vitamin B₁₂ without any additional substances. In iron deficiency he uses only iron. He does not believe that the various factors in B-Complex, singly have any effect on human anemia. Used in combination, in anemias, they may upset the enzyme equilibrium of the body. Liver extract, crude or refined, still remains a potent method of avoiding this accident while, at the same time, improving the anemia."

- 234 HECK, F. J.: *Proper use of iron, liver extract, vitamin B₁₂, and folic acid in anemias* J.A.M.A. 148 783-788, March 8, 1952.

Author's summary "Except possibly in some very unusual situation the treatment of anemia resolves itself into the use of specific substances depending on the morphologic blood picture. In hypochromic anemias, which are usually due to loss of blood, the proper treatment is the oral use of a preparation of iron. For these anemias, liver extract, vitamin B₁₂, folic acid, desiccated hog stomach, and stomach-liver or iron-stomach-liver preparations serve no useful purpose and are definitely not indicated. In those unusual instances in which iron taken by mouth cannot be tolerated or produces an increase in gastrointestinal symptoms, it can be given intravenously."

"The commonest diagnosis of macrocytic anemias with megaloblastic type of bone marrow in northern United States is pernicious anemia. The most desirable form of treatment of this type of anemia is the parenteral use of a potent liver extract or the parenteral use of vita

min B₁₂. Preparations of liver or stomach or liver-stomach combinations for oral administration can be used and are effective when given in adequate amounts. The presence of neurologic manifestations with pernicious anemia requires that much larger quantities of potent material be used either parenterally or orally. Iron is not indicated unless loss of blood is associated with pernicious anemia. Folic acid, while not contraindicated, is not necessary in the complete treatment of pernicious anemia and when used alone, constitutes inadequate treatment. (Experience with citrovorum factor is insufficient as yet for anyone to know whether it will merely react on a megaloblastic marrow and convert it to normal and then, as in the case of folic acid, fail to prevent gastrointestinal and neurologic manifestations and permit eventual relapse of hematologic symptoms. The author's limited experience with citrovorum factor confirms the good hematologic response recently reported.)

"Concerning other diseases associated with megaloblastic anemia, there are differences of opinion regarding the response to vitamin B₁₂ and folic acid. Some patients will respond only partially or not at all to vitamin B₁₂, and subsequently a good result can be obtained with folic acid.

"None of the substances discussed in this paper are of value in the treatment of the anemia associated with (1) various types of malignant lesions, (2) diseases of the reticuloendothelial system, and (3) diseases in which there is toxemia or interference with metabolism of hemoglobin.

"Treatment with iron, liver extract, vitamin B₁₂, or folic acid is of benefit only when these substances are deficient, and none of these forms of medication is helpful in anemia due to some cause other than that produced by these specific deficiencies. A useful maxim then is: Determine the type of anemia present, give only the medication indicated, do not mix the treatment." Fifteen references are cited.

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- 235 REISNER, E. H., Jr. *The pathogenesis of anemia as a basis for rational treatment*. New York State J. Med. 52 1645-1648, July 1, 1952.

An initial hemogram greatly facilitates diagnosis of the type of anemia. If the bone marrow is not functioning, the color index will suggest the cause. Nutritional macrocytic anemia and sprue respond to vitamin B₁₂ or folic acid. In pernicious anemia, folic acid apparently speeds up reactions that exhaust the already depleted stores of vitamin B₁₂ and may aggravate the neurologic lesions of the disease; therefore vitamin B₁₂ should be used in such cases. Megaloblastic anemia of infancy and pernicious anemia of pregnancy respond best to folic acid.

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236. UNGLEY, C. C. *Clinical and laboratory aspects of vitamin B₁₂*. *Lancet* 2 134, July 19 1952 (In Soc. Proc.) *Vitamin B₁₂*. *Brit. M. J.* 2 154 July 19 1952 (In Soc. Proc.)

It is essential to differentiate between megaloblastic due to lack of vitamin B₁₂ and that due to lack of folic acid. Anemias involving dietary deficiency have been benefited by vitamin B₁₂ therapy. A case is cited in which a patient with anemia subsequent to a total gastrectomy responded well to oral administration of 1,000 mcg. of the vitamin.

The fish-tapeworm anemia of Finland has been attributed to some toxin in the worm; its dried body however has been found to be "a rich source of vitamin B₁₂," and was curative when orally administered. This observation suggests that the living worm takes up vitamin B₁₂ in the intestine.

Harrold-ayres-Tyoe, England

- 237 RHEINGOLD, J. J. *The treatment of the anemias*. M. Ann. District of Columbia 21 368-371, July 1952.

The need for accurate diagnosis of the cause of anemia is stressed. Anemia resulting from deficiency of the erythrocyte maturing factor or the so-called anti-pernicious anemia factor is treated with refined liver extract, vitamin B₁₂, or folic acid. In pernicious anemia, liver extract or vitamin B₁₂ is necessary to prevent the development of neurologic symptoms, and combining such treatment with folic acid does not seem to produce better results. In sprue, however combined therapy with liver or B₁₂ and folic acid seems to give better results than does either of these agents alone. Pernicious anemia of pregnancy and of infancy seem to respond better to folic acid than to either vitamin B₁₂ or liver extract. Atypical megaloblastic anemia responds sometimes to one and sometimes to another agent in some instances a combination may be necessary.

The recommended dosage of vitamin B₁₂ in pernicious anemia-like diseases is 50 mcg. a day intramuscularly for a patient whose red blood cell count is approximately 1,000,000. When a maximum reticulocyte response has been gained, this dose may be given every other day and later at longer intervals until eventually an injection of 50 mcg. once a month will suffice.

In anemias involving disturbance of the bone marrow supportive therapy is helpful, and occasionally corticotropin or cortisone will result in the formation of normal bone marrow. When splenomegaly is involved, splenectomy is usually the treatment of choice. In cases where there is no response, corticotropin or cortisone may be helpful in obtaining a remission. These hormones have also been used successfully by some investigators in acquired hemolytic anemia, and the author believes they should be tried before splenectomy is resorted to.

Iron deficiency anemias, and anemias resulting from blood loss or destruction are also discussed.

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238. BETHELL, F. H. *Treatment of anemia*, Wisconsin M. J. 51 1062-1064, Nov 1952.

Accurate classification as to type of anemia is a primary requisite. Complex mixtures of vitamins, other hematinics as proprietary preparations

have no place in treatment. Specific therapy includes measures to supply hemopoietic factors which may be missing (iron, folic acid, vitamin B₁₂, ascorbic acid, dietary amino acids) and to diminish excessive rate of blood destruction.

Microcytic hypochromic anemia is treated with iron-containing foods or iron orally or intravenously.

The pathogenesis of macrocytic anemias has been elucidated in recent years. It is now known that both folic acid and vitamin B₁₂ are required for normal hemopoiesis and that they participate in widespread metabolic processes. Folic acid is widely distributed in foods, but chiefly in conjugated forms that require enzymatic action for the release of the free vitamin, which is believed to be the only form that can be absorbed. Thus, folic acid deficiency may result from failure of conjugase action. Evidence that the biologically active form of folic acid is folinic acid has been obtained: ascorbic acid facilitates the conversion of folio to folinic acid in living tissues. Ascorbic acid plays a part in hemopoiesis both in metabolism of folic acid and in utilization of iron. Appreciable amounts of vitamin B₁₂ may become available through bacterial synthesis, but intestinal bacteria that require an exogenous source of this vitamin for growth may utilize it to the deprivation of the host. Intrinsic factor may combine with the extrinsic factor (vitamin B₁₂) forming a microbiologically inactive complex; this may facilitate absorption, perhaps by preventing utilization by coliform organisms. In pernicious anemia the primary factor appears to be lack of intrinsic factor resulting in a conditioned vitamin B₁₂ deficiency. Dietary lack of folic acid appears to be a more common cause of nutritional macrocytic anemia than lack of B₁₂. Chronic intestinal disorders (idiopathic steatorrhea, disseminated ileitis, partial obstruction, diminished absorptive surface) may impair resorption of both folic acid and B₁₂, resulting in the neurologic changes seen in true pernicious anemia.

Specific measures available for treatment of macrocytic megaloblastic anemias include liver extract, deacidulated stomach, vitamin B₁₂ and folic acid. Vitamin B₁₂ usually constitutes a complete form of replacement therapy for pernicious anemia; for macrocytic megaloblastic anemias other than pernicious, folic acid orally (parenterally if intestinal disease is present) or B₁₂ orally if pernicious anemia can be excluded, will usually correct dietary deficiency of these vitamins.

In hemolytic anemias the treatment used in anemia caused by defective blood formation is ineffective. Corticotropin and cortisone have proved of real merit in idiopathic acquired hemolytic anemia; they may terminate an acute crisis, permit reasonably safe splenectomy or even induce long-lasting complete remissions without other therapy or need of continuous administration of the hormones.

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- 239 LUTTGENS, W. F. *The newer hematinases, their use and abuse*, California Med. 77: 369-373, Dec. 1952.

In a review based on 51 published articles the author discusses the action of vitamin B₁₂, folic acid, citrovorum factor and saccharated oxide of iron given intra-

venously. Vitamin B₁₂ appears to be the active principle of refined liver extract and is effective in pernicious anemia when given alone parenterally or with a source of intrinsic factor such as normal gastric juice, by mouth. Folic acid usually brings about complete remission in the other types of megaloblastic anemia, and these remissions may be permanent notwithstanding cessation of therapy.

Because folic acid can obscure changes in the blood and thus prevent early recognition of pernicious anemia, in turn permitting neurologic disease to develop, inclusion of folic acid in polyvitamin preparations is decried. Saccharated iron is relatively safe for intravenous administration but is of value only in iron-deficiency hypochromic anemia, and is seldom indicated. Overdosage should be avoided because the body cannot excrete iron.

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- 240 SPIES, T. D., STONE, R. E., SUAREZ, R. M., GARCIA LOPEZ, G., LOPEZ-TOCA, R., and REBOREDO A.: *Anisemic properties of reaction products of vitamin B₁₂ and the intrinsic factor* J.A.M.A. 151: 1264-1266, April 11, 1953.

Patients with pernicious anemia vary greatly in their requirements for vitamin B₁₂. Some patients respond to small oral doses of vitamin B₁₂ without intrinsic factor and some respond only if a source of intrinsic factor is given concomitantly.

The authors investigated potent intrinsic factor preparations from several manufacturers and found that these potentiate the effect of vitamin B₁₂ in pernicious anemia, nutritional macrocytic anemia and tropical sprue. The effect of one proprietary preparation, tablets of which contain active intrinsic factor and vitamin B₁₂, was studied in 11 patients with pernicious anemia, 1 with nutritional macrocytic anemia and 2 with tropical sprue. These patients had not shown a hematologic response to 10 to 15 mcg. vitamin B₁₂ orally (daily?) during a 10 to 30 day control period. The dosage of vitamin B₁₂ obtained in this proprietary never exceeded that given during the control period. All 11 patients responded hematologically and clinically.

Three patients who were known not to respond to oral vitamin B₁₂ even when it was given together with human gastric juice, also failed to respond to this intrinsic factor preparation. They responded well to a single intramuscular injection of 10 mcg. of vitamin B₁₂.

A comparative evaluation was made of the responses of 11 cases of pernicious anemia in relapse and 11 cases of tropical sprue in relapse. This was done by finding an amount of intrinsic factor concentrate that, when administered with 10 mcg. of vitamin B₁₂, would produce a definite but submaximal clinical and hemopoietic response in the cases of pernicious anemia. These concentrates were then tested in the patients with tropical sprue. None of the sprue patients had any clinical or hemopoietic responses, thus showing that the amount of vitamin B₁₂ and intrinsic factor that will produce a definite but submaximal response in the average case of pernicious anemia is inadequate in the average case of tropical sprue.

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have no place in treatment. Specific therapy includes measures to supply hemopoietic factors which may be missing (iron, folic acid, vitamin B₁₂, ascorbic acid, dietary amino acids) and to diminish excessive rate of blood destruction.

Microcytic hypochromic anemia is treated with iron-containing foods or iron orally or intravenously.

The pathogenesis of macrocytic anemias has been elucidated in recent years. It is now known that both folic acid and vitamin B₁₂ are required for normal hemopoiesis and that they participate in widespread metabolic processes. Folic acid is widely distributed in foods, but chiefly in conjugated forms that require enzymatic action for the release of the free vitamin, which is believed to be the only form that can be absorbed. Thus, folic acid deficiency may result from failure of conjugase action. Evidence that the biologically active form of folic acid is folinic acid has been obtained; ascorbic acid facilitates the conversion of folate to folinic acid in living tissues. Ascorbic acid plays a part in hemopoiesis both in metabolism of folic acid and in utilization of iron. Appreciable amounts of vitamin B₁₂ may become available through bacterial synthesis, but intestinal bacteria that require an exogenous source of this vitamin for growth may utilize it to the deprivation of the host. Intrinsic factor may combine with the extrinsic factor (vitamin B₁₂) forming a microbiologically inactive complex; this may facilitate absorption, perhaps by preventing utilization by coliform organisms. In pernicious anemia the primary factor appears to be lack of intrinsic factor resulting in a conditioned vitamin B₁₂ deficiency. Dietary lack of folic acid appears to be a more common cause of nutritional macrocytic anemia than lack of B₁₂. Chronic intestinal disorders (idiopathic steatorrhea, disseminated sclerosis, partial obstruction, diminished absorptive surface) may impair resorption of both folic acid and B₁₂, resulting in the neurologic changes seen in true pernicious anemia.

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239 LUTTGENS, W. F.: *The new and old*, California Med. 1952.

In a review based on 51 published articles that discusses the action of vitamin B₁₂, folic acid and saccharated oxide of iron.

venously. Vitamin B₁₂ appears to be the active principle of refined liver extract and is effective in pernicious anemia when given alone parenterally or with a source of intrinsic factor such as normal gastric juice, by mouth. Folic acid usually brings about complete remission in the other types of megaloblastic anemia, and these remissions may be permanent notwithstanding cessation of therapy.

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San Francisco, Calif.

240. SPIES, T. D., STONE, R. E., SUAREZ, R. M., GARCIA-LOPEZ, G., LOPEZ-TOCA, R., and REBOREDO, A. Antianemic properties of reaction products of vitamin B₁₂ and the intrinsic factor. J.A.M.A. 151: 1264-1266, April 11, 1953.

Patients with pernicious anemia vary greatly in their requirements for vitamin B₁₂. Some patients respond to small oral doses of vitamin B₁₂ without intrinsic factor and some respond only if a source of intrinsic factor is given concomitantly.

The authors investigated potent intrinsic factor preparations from several manufacturers and found that these potentiate the effect of vitamin B₁₂ in pernicious anemia, nutritional macrocytic anemia and tropical sprue. The effect of one proprietary preparation, tablets of which contain active intrinsic factor and vitamin B₁₂, was studied in 8 patients with pernicious anemia, 1 with nutritional macrocytic anemia and 2 with tropical sprue. These patients had not shown a hematologic response to 10 to 15 mcg. vitamin B₁₂ orally (daily?) during a 10 to 80 day control period. The dosage of vitamin B₁₂ obtained in this proprietary never exceeded that given during the control period. All 11 patients responded hematologically and clinically.

Three patients
oral vitamin
B₁₂

not to respond to
together with
to this intrinsic
factor as a single

ANALYTICAL STUDIES

- 241 JACOBSON B. M., and BISHOP R. C. *Studies of the principle in liver effective in pernicious anemia. III The augmentative effect of accessory factors on the therapeutic activity of vitamin B₁₂*. J. Clin. Investigation 28 791 July 1949 (In Soc. Proc.)

The therapeutic action of liver extract in pernicious anemia depends upon the presence of a primary factor which exerts only a slight or moderate hemopoietic effect by itself but is active in the presence of five chemically distinct accessory factors (L-tyrosine, a peptide, xanthopterin, tryptophane, guanidine). These accessory factors are inert without the primary factor. Erythrocyte regeneration curves constructed from data in the literature and from personal observation of 16 patients who received a calculated average daily dose of 2 mcg. of vitamin B₁₂ by parenteral injection were compared with similar curves showing the effect of commercial liver extract in the calculated daily dose of 2.1 U.S.P. units. The average daily increment of erythrocytes in a range of initial erythrocyte levels from 1.50 to 1.99 millions per cu. mm. was 78 for commercial liver extract, 86 for primary factor with accessory factors, and 59 for vitamin B₁₂. In a range from 2.5 to 2.99 millions per cu. mm., the erythrocyte increments were 63, 61, and 31 respectively. The augmentative effect of accessory factors on the action of vitamin B₁₂ is illustrated by data on individual cases.

242. LICHTMAN H., WATSON J., GINSBERG V., PIERCE J. V., STOKSTAD E. L. R., and JUKES T. H.: *Vitamin B₁₂ some properties and its therapeutic use*, Proc. Soc. Exper. Biol. & Med. 72 643-645, Dec. 1949

The authors have obtained a pink fraction from concentrated liver extract by chromatography with silicic acid. Upon purification, this fraction yields a red pigment with an absorption spectrum differing from that of vitamin B₁₂. An apparently similar fraction is obtained from cultures of *Streptomyces aureofaciens*, and crystals prepared from this fraction have been used in the treatment of 5 patients with Addisonian pernicious anemia. Intramuscular injection of 1.0, 1.5, or 2.0 mcg. a day in saline solution, gave an optimal hemopoietic response. The reticulocyte peaks obtained by this treatment varied, but in all 5 patients the erythrocyte regeneration was within maximal limits at the end of 21 days of therapy. In the single patient who was treated for more than three weeks, the red cell count was nearly normal at the end of seven weeks. Clinical improvement was also satisfactory.

In a sixth patient with Addisonian pernicious anemia, the oral administration of 5 mcg. of vitamin B₁₂ for 10 days was ineffective, but when neutralized gastric juice was added, the reticulocytes began to rise slowly on the eighth day.

One patient with megaloblastic anemia of pregnancy showed no hematologic response to 1 mcg. of vitamin B₁₂ a day intramuscularly but her severe glossitis cleared completely. Later she showed a maximal hematologic response to pteroylglutamic acid.

Flour County Hospital
Anchorage, N. Y.
Lederle Laboratories
Pearl River, N. Y.

- 243 LAJTHA, L. G.: *An inhibitory factor in pernicious anemia serum*. Clin. Sc. 9 227-277 1950.

The factor in pernicious anemia serum which inhibits cell maturation is thermostable and can be overcome in vitro by folic acid or liver extract but not by vitamin B₁₂.

- 244 KJERULF JENSEN K., and NOER, B.: *Failing effect on pernicious anemia of liver extracts inactivation of their vitamin B₁₂ active material*, Acta pharmacol. et tox. 6 92-96, 1950.

After exposure to the light of an ordinary electric lamp a commercial liver extract lost its characteristic therapeutic effect toward pernicious anemia together with its content of microbiologically active vitamin B₁₂ material. (Autoclaving at pH 10 and 121° for 1 hour gave a similar effect.)

The complete failure in efficacy of the liver extract after inactivation of vitamin B₁₂ here recorded speaks in favor of the conception of vitamin B₁₂ as the predominant substance of liver extracts.

These results indicate that the clinical effect is proportional to the microbiologic B₁₂ activity and that a liver extract without vitamin B₁₂ content has no clinical effect.

Furthermore the extreme instability of vitamin B₁₂ in liver extracts exposed to light indicates that the relationship between clinical response and microbiological activity must be investigated on the same sample or on samples that have been kept under exactly the same conditions.

BLOOD CELLS

245. DEDICHEN J., and LALAND P.: *Anti-pernicious anemia factor and white-cell count*, Lancet 2 282-283, Aug. 13, 1949

In a normal individual, the leukocyte count was uninfluenced by the intramuscular injection of 40 mcg. of pure crystalline anti-pernicious anemia factor but rose significantly following intramuscular administration of 10 cc. of a potent liver extract. One of the authors had previously noted that folic acid in doses of 25 mg. intramuscularly had no effect on the white cell count of normal subjects. These findings indicate that the leukocytosis factor in liver extract reported by Dedichen and fractionated by Laland several years ago is not identical with the pure anti-pernicious anemia factor or with folic acid.

The University Hospital, and
Brogard & Co.
Oslo, Norway

246. OCKRENT C. *Relation between vitamin B₁₂ and the red blood cells* Nature 165 220-221 Feb. 18 1950 (In Letters to the Editor)

A hypothesis is presented to explain the therapeutic action of vitamin B₁₂ in pernicious anemia in what is commonly considered surprisingly small doses. The number of B₁₂ molecules in a 10 mcg. dose is calculated 5x10¹⁴. The average total number of red blood in an adult human in good health is estimated. The number of red blood cells may number or less in a case of pernicious anemia.

It is apparent that there are more than enough B_{12} molecules for a one-to-one correspondence with the red blood cells and that an excess of B_{12} is available for storage in the liver. On B_{12} injection, a rapid rise is observed in the number of reticulocytes, the precursors of the erythrocytes. It therefore appears probable that at least one B_{12} molecule interacts, at an earlier stage of the normoblast process, with one blood cell. It may be that the B_{12} molecule provides in some way an essential unit for each individual blood cell in an early stage of its development.

The British Drug Houses, Ltd.
London, England

- 247 JONES, D. P., and SMITH, A.: *Transmission of antianemic principle across the placenta and its influence on embryonic erythropoiesis. II Comparison of the effect of liver extract and pteroylglutamic acid (PGA)* Blood 5: 499-521, June 1950.

Both liver extract and PGA (folic acid) cause a reduction in mean cell diameters, but the former is slightly more effective.

248. LARSEN O.: *Vitamin B₁₂*, Lancet 2: 407 Sept. 23, 1950 (In Soc. Proc.)

"Dr O Larsen (Norway) described the occurrence in pernicious anaemia blood of large thin cells which he called macrocytes—in contradistinction to megaloocytes which are large cells of normal thickness. It is these cells that cause macrocytosis persisting long after the mean cell volume becomes normal under treatment; they have a life-span of 40-60 days, and a considerable proportion of their haemoglobin appears to be foetal. Treatment with vitamin B_{12} , folic acid, and some commercial liver extracts fails to influence the macrocytes, and Dr Larsen suggested that liver contains another factor necessary to produce a completely normal blood picture. This factor had been separated and is not haemopoietic in the usual sense."

- 249 WATSON C. J.: *The erythrocyte coproporphyrin variances in respect to erythrocyte protoporphyrin and reticulocytes in certain of the anemias*, Arch. Int. Med. 85: 797-809 Dec. 1950.

In addition to the protoporphyrin previously studied, human erythrocytes contain free coproporphyrin normally not exceeding 2 mcg. per 100 cc. of red blood cells. Studies of the relationship between erythrocyte protoporphyrin, erythrocyte coproporphyrin, and reticulocytes in various anemias, especially in pernicious anemia during the response to vitamin B_{12} therapy suggest that the erythrocyte coproporphyrin value is a rather sensitive chemical index of the rate of hemoglobin synthesis (or attempted synthesis) in the bone marrow

University of Minnesota Hospital
Minneapolis, Minn.

250. MUEHRKE, R. C., and KARK, R. M.: *Negative effects of vitamin B₁₂ on blood of healthy individual male, with special reference to eosinophils*, Proc. Soc. Exper. Biol. & Med. 77: 144-145, May 1951.

In patients with pernicious anemia who responded to vitamin B_{12} therapy the authors were surprised to find no increase in eosinophils, such as occurs when liver ex-

tract or whole liver is administered to healthy individuals or to patients with pernicious anemia. Studies were made of the blood of 8 healthy subjects (3 adults and 5 children) who were given large amounts of vitamin B_{12} for 28 days. The adults were given 300 mcg. a day orally or 75 mcg. parenterally; the children received 10 mcg. a day orally in 4 cases and intramuscularly in the fifth case. The blood of these subjects was not affected. In particular no eosinophilia was observed. Neither allergic nor toxic symptoms were noted during or after therapy. The authors conclude that vitamin B_{12} is not the eosinophilic factor present in raw calves' liver

University of Illinois College of Medicine, and
Research and Educational Hospitals
University of Illinois
Chicago, Ill.

251. BENARD H., GAJDOS, A., and GAJDOS-TÖRÖK, M.: *Action of vitamin B₁₂, folic acid and liver extracts on the free protoporphyrin content of red blood cells* Nature 167: 990-991 June 16, 1951 (Letter to the Editors)

In secondary anemias and in iron deficiency anemia, the free protoporphyrin is increased while in Addison's anemia it is decreased. Experiments with rabbits indicate that agents used in the treatment of pernicious anemia, such as vitamin B_{12} , folic acid, and active liver extracts, produce a considerable increase in the free protoporphyrin content of the erythrocytes. This increase of free protoporphyrin is attributed to the acceleration of the synthesis of the pigment. Rabbits that had been depleted of glycine, which is used in building porphyrin, did not show an increase in free protoporphyrin when injected with vitamin B_{12} or folic acid. When glycine was given again, the normal response to vitamin B_{12} or to folic acid was reestablished.

These investigations demonstrate a new aspect of the mechanism of action of agents effective in the treatment of pernicious anemia. The authors believe that vitamin B_{12} , folic acid, and active liver extracts act, at least in part, in pernicious anemia by restoring the synthesis of protoporphyrin, a synthesis which is defective in this disease.

Chimie Médicale du Pitié-Salpêtrière
Paris, France

252. LEVEY S., and ORTEN, J. M.: *Vitamin B₁₂ and production of polycythemia by cobalt*, J. Nutrition 45: 487-492, Dec. 1951.

Cobalt does not produce a polycythemia by way of the intermediary formation of vitamin B_{12} .

- 253 THOMPSON R. B.: *Observations on the effects of vitamin B₁₂, liver extracts, folic acid and thymine on the maturation of megakaryoblasts in culture*, Blood 7: 522-525, May 1952.

Author's summary "Folic acid has been demonstrated to have a direct maturing effect on cultures of megakaryoblasts obtained from patients suffering from pernicious anemia in relapse. No effect has been observed with either liver extracts, vitamin B_{12} [10 to 100 mcg./cc.] or thymine."

Royal Free Hospital Laboratory
Newcastle-on-Tyne, England

- 254 HAMILTON H. E., SHEETS R. F., and JANNEY C. D.: *A hemolytic mechanism in pernicious anemia influenced by B₁₂*, J. Clin. Investigation 31 636, June 1952 (in Soc. Proc.)

"Eight patients with pernicious anemia were transfused with fresh blood from normal donors of heterologous, compatible A-B-O group and Rh type. The concentrations of donor erythrocytes during periods up to 120 days were measured by serial counts of inagglutinable cells by a modification of Ashby's technique yielding accurate data for mathematical analysis.

"Analysis of the disappearance curves of normal transfused cells showed normal survival with B₁₂ started 9 to 12 days before transfusion. In patients beginning treatment 9 days before and 1, 4, and 5 days after transfusion, random destruction of the normal erythrocytes, irrespective of age, was demonstrated. In general, the rate of loss increased the later B₁₂ was given, with the fastest rate in the untreated patient.

"In six studies the rate of random cell destruction was constant throughout the four month period, despite coincidentally adequate reticulocyte responses and satisfactory rises in total erythrocyte counts. In two studies exhibiting the fastest rate of destruction the rate accelerated about 70 days after transfusion.

"Conclusions: (1) Fresh normal erythrocytes are so damaged in a few days in the milieu of the human body deficient in B₁₂ that they are destroyed in random fashion, presumably by the trauma of circulation. (2) The cell damage incurred in the first few days is not reversible by the subsequent administration of B₁₂. (3) When severe grades of erythrocyte damage have occurred, the chemical changes of aging or the trauma of 70 days of circulation further hasten destruction. (4) These studies indicate that 45 micrograms of B₁₂ weekly must be present in the body for at least 10 days before a safe environment is provided for normal erythrocytes. Until further evidence is available, it is assumed that the hemolytic mechanism exerts similar effects on the circulating erythrocytes of the patient."

Leona Cary Jones

255. DEGOWIN, E. L., HAMILTON H. E., SHEETS R. F., JANNEY C. D., and ELLIS J. A.: *The role of maturation arrest in the low erythrocyte counts of pernicious anemia*, J. Lab. & Clin. Med. 40 790-791, Nov 1952 (in Soc. Proc.)

"Fresh normal erythrocytes belonging to group O were transfused to six patients with pernicious anemia in relapse belonging to group A or B. Donor cells were identified in the circulation of the patients by Ashby's method of differential agglutination. Analysis of inagglutinable erythrocyte counts demonstrated that the normal donor's erythrocytes were so affected by twenty four hours or more in the contact with a deficiency of B₁₂ in the body of the patients that the transfused cells were randomly destroyed at rates that could be measured rather accurately.

"At the beginning of the transfusion experiments the erythrocyte levels of the patients were in steady states with counts of 1.0, 1.64, 1.70, 2.00, 2.00, and 3.5 millions

per cubic millimeter. In each the aspirated bone marrow presented the cytologic picture which has been considered typical of 'maturation arrest'. The subsequent administration of B₁₂ parenterally resulted in prompt rises of the patients' erythrocyte counts to normal and disappearance of all symptoms and signs of the disease except those attributed to permanent changes in the spinal cord.

"Assuming a normal rate of erythropoiesis when the cells are being destroyed by a hemolytic mechanism, the resulting lowered level of the erythrocyte count can be calculated, if the rate of destruction is known. In these studies it was assumed that the rates of destruction of the patient's erythrocytes were similar to the measured rates at which the biologically tagged cells disappeared.

"In four patients the observed levels of erythrocyte counts before treatment coincided with those calculated on the assumption of normal erythropoietic rates and the observed random loss of donor's cells. The lowered cell counts could therefore be explained completely by the action of a hemolytic mechanism and without maturation arrest of significant magnitude. In the other two patients this method of calculation suggested that about one-half of the lowering of the erythrocyte count could be attributed to a hemolytic mechanism, whereas the other one-half was explainable by a phenomenon such as maturation arrest. Reticulocyte response to B₁₂ was significantly lower than expected in those in which the hemolytic mechanism predominated."

- 256 JANNES, J.: *Studies on the vitamin B₁₂ content of human blood plasma*, Ann. med. exper. et biol. Fenniae 80 Fasc. 3-4 329-1952 (abstr. J. Am. Dietet. A. 29 158, 160, Feb. 1953)

"Results are given of studies of the vitamin B₁₂ level of the blood plasma of five healthy persons and of two patients with pernicious anemia. The method by which the determinations were made is described."

BONE MARROW

- 257 SIGNY A. G.: *Recent work on vitamin B₁₂*, Lancet 1: 354-355, Feb. 25, 1950 (in Soc. Proc.)

At a meeting of the section of experimental medicine of the Royal Society of Medicine, Dr. Signy "reported what he thought was an effect on the bone-marrow one hour after injection [of B₁₂] in a patient in whom the marrow was studied hourly."

258. MOLLIN D. L., and DACIE, J. V.: *Recent work on vitamin B₁₂. Observations on the relationship between the red cell and reticulocyte responses and changes in the bone-marrow of patients suffering from pernicious anaemia treated with injections of liver extracts or vitamin B₁₂*, Proc. Roy. Soc. Med. 43 541-546, July 1950

- 259 DACIE, J. V.: *Recent work on vitamin B₁₂*, Lancet 1: 354, Feb. 25, 1950 (in Soc. Proc.); Proc. Roy. Soc. Med. 43 541-546, July 1950.

The author has described "30 cases of true pernicious anaemia treated with liver extract, vitamin B₁₂ and Lester Smith's unnamed substance. It was a

finer relations between the histological picture of the bone-marrow the size of the reticulocyte response, and the increase in the red cells. With a single dose of 20 μ g. of vitamin B₁₂ the marrow became normoblastic in the first five days, and reticulocytes might account for nearly all the red-cell increase. In some cases the marrow reverted to an intermediate stage as early as the 5th day and was megaloblastic by the 10th day. Dr Dacie found that the red-cell response was biphasic, with a shelf between the 5th and the 9th day. During the second rise there were fewer reticulocytes, and almost all the increase was due to cells already mature as they reached the bloodstream. The size of the second rise depended on the dose and could be correlated with the marrow picture. A single initial dose of 80-40 μ g. caused the marrow to remain normoblastic to the 15th day; 20 μ g. gave a satisfactory response in the blood but allowed a regression of the marrow to the megaloblastic stage in the same period."

250. MAGNUSSEN J D: *L'influence de l'extrait de fœtus et de la vitamine B₁₂ sur la production des érythrocytes in vitro* (The influence of liver extract and vitamin B₁₂ on the production of erythrocytes in vitro) Acta pharmacol. et toxicol. 6: 263-266, 1950 (abstr. Semaine d. hóp. de Paris 27: 107 Jan. 6, 1951)

Vitamin B₁₂ can completely replace liver extract as a nutritive element for the cell lines of the marrow in the study of the development of erythrocytes in vitro. It is probable that the effect of the liver extract is due to its vitamin B₁₂ content. Probably vitamin B₁₂ does not have the same action in vivo, for the concentrations used in vitro exceed those that can be obtained in the blood. These results were obtained partly by spectrographic study and partly by study of the growth of *Lactobacillus* Dorner

251. FADESI, R. S., and BERLIN, I: *Comparisons between bone marrow differentials prepared from particles and from random samples of aspirate and determinations of the dilution of aspirate with peripheral blood utilizing radioactive phosphorus (P³²)* Blood 11: 160-174, Feb. 1952.

In an article in which bone marrow differentials are compared, a case of pernicious anemia in relapse is described. The patient was successfully treated with parenteral vitamin B₁₂ after oral vitamin B₁₂ had been ineffective.

From the Laboratory of Hematology, H. Hospital, Boston, U. S.

252. EDITORIAL: *Vitamin B₁₂ in the bone marrow* South. M. J. 44: 361-362, April 1951.

The results obtained by Horrigan, Jarrold, and Vilter who studied the effects of folic acid and vitamin B₁₂ when instilled into the marrow of patients with pernicious anemia in relapse (*J. Clin. Investigation* 30: 81, Jan. 1951) are summarized. Folic acid was ineffective when administered thus; it probably requires alteration inside the body before it can stimulate the bone marrow. Unaltered by stomach or liver corrects a qualitative abnormality in cellular ribonucleic acid of patients with pernicious anemia in relapse. Both folic acid and B₁₂ are

believed to be concerned in the formation of nucleic acid. In patients with pernicious anemia in relapse, ribonucleic acid is thought to be abnormally distributed, and this is corrected by vitamin B₁₂. Both folic acid and B₁₂ correct abnormal erythrocyte maturation, the first only after a structural change in the body the second by direct reaction with the marrow components after injection into the marrow cavity.

253. ETESS, A. D., and LITWINS J: *Pernicious anemia with early marrow change following therapy* New York State J. Med. 51: 2787-2788, Dec. 1 1951.

After the diagnosis of pernicious anemia had been made in a patient, a sternal marrow needle was inserted and marrow was aspirated. Following the administration of 60 mcg. of vitamin B₁₂ intramuscularly marrow specimens were withdrawn every 15 minutes for three hours, and daily for three days. Within two and a half hours there was a decrease in megaloblasts (from 15 to 1.8%) and a corresponding increase in normoblasts (from 49 to 74%). Peripheral smears showed no change in the size of the erythrocytes, and the reticulocytes did not increase. It is pointed out that after the administration of vitamin B₁₂, a diagnosis of pernicious anemia cannot be made with certainty. Liver extract or vitamin B₁₂ should not be given until a diagnosis is made, to prevent the masking of the signs of the disease.

From Cornell Medical School, New York, N. Y.

254. STRAUSS, M. B., BROKAW R., and CHAPMAN C. B.: *Leukemoid bone marrow in pernicious anemia*, Am. J. M. Sc. 223: 54-60, Jan. 1952.

Authors' summary and conclusions: "Two patients with Addisonian pernicious anemia complicated by infection presented bone marrow pictures characterized by intense granulocytic activity and almost devoid of megaloblasts. In each the diagnosis of aleukemic myelogenous leukemia was considered. Both responded uneventfully to vitamin B₁₂ therapy and subsequently exhibited entirely normal marrow. It is concluded that the bone marrow in pernicious anemia in relapse is not always pathognomonic."

In each case the first dose of vitamin B₁₂ was 45 mcg. One patient received a maintenance dose of 15 mcg. every week, and the other received 45 mcg. every week.

From the Department of Hematology, H. Hospital, Boston, U. S.

From the Laboratory of Hematology, H. Hospital, Boston, U. S.

CELL TYPES, CONTENTS

255. HERRIGAN, D., and VILTER, R. W.: *Direct action of vitamin B₁₂ upon human bone marrow: the effect of instillations of vitamin B₁₂ and folic acid into the bone marrow as studied by histochemical techniques* Program 42nd Annual Meeting, Am. Soc. Clin. Investigation, May 1, 1950, p. 83 (advance abstr.); J. Lab. & Clin. Med. 29: 623, June 1950 (In Soc. Proc.)

In 5 patients with megaloblastic anemia, 1 mcg. of vitamin B₁₂ or 1 mg. of folic acid was injected into one

iliac crest marrow cavity and 40 hours later marrow was aspirated from the same site and also from the opposite iliac crest. From the findings the authors conclude that vitamin B₁₂ can be utilized locally by bone marrow cells and it corrects a qualitative abnormality in cellular ribonucleic acid of persons with pernicious anemia. folic acid, however, cannot be utilized locally and probably must be converted to an active hematopoietic substance by enzymatic activity elsewhere in the body.

Chickens, Ohio

256. HERRIGAN D., JARROLD T., and VILTER, R. W. Direct action of vitamin B₁₂ upon human bone marrow: the effect of instillations of vitamin B₁₂ and folic acid to the bone marrow as studied by nucleic acid staining techniques. *J Clin Investigation* 30 31-36, Jan. 1951

The action of vitamin B₁₂ and of folic acid upon human bone marrow was studied in 6 persons with pernicious anemia in relapse. Three received injections of 1 cc. of solution containing 1 mcg. of vitamin B₁₂ and 3 received injections of 1 cc. of solution containing 1 or 2 mg. of folic acid. In each case the injection was made into the marrow cavities of the iliac crests after aspiration of 2 to 3 cc. of marrow and blood. Forty-eight hours later bone marrow was aspirated from the exact site of the previous instillation and also from the opposite iliac crest. The aspirated specimens were stained to permit differential cell counts and demonstration of cytoplasmic ribonucleic and deoxyribonucleic acids.

It was found that vitamin B₁₂ can be utilized locally by the bone marrow cells and corrects a qualitative abnormality in cellular ribonucleic acid in persons with pernicious anemia in relapse. It need not be altered by stomach or liver to exert this effect. Folic acid is not utilized locally by bone marrow within 48 hours after instillation into the marrow cavity but when given orally or parenterally it has the same cytologic and cytochemical effects as vitamin B₁₂. It is probable that folic acid must be converted to an active hematopoietic substance by enzymatic activity elsewhere in the body.

University of Cincinnati College of Medicine
Cincinnati, Ohio

257. HERRIGAN D. L., and HEINLE, R. W. Role of tissue binding in local erythroid maturation following instillation of vitamin B₁₂ into marrow cavity. *J Clin. Investigation* 31 689 June 1952 (in Soc. Proc.)

"A local erythroid maturation effect following instillation of vitamin B₁₂ directly into marrow cavity of persons with pernicious anemia was described previously. The interpretation of this observation was that the vitamin was capable of inducing maturation without first being altered.

"Other investigators have shown that vitamin B₁₂ exists in the blood in bound form that many substances are capable of binding, and that the unbound vitamin is ineffective in causing erythroid maturation in vitro.

"In this study the observations indicate that the vitamin is bound at the site of instillation but that binding capacity is limited. After instillation of 1 mcg. into the

marrow cavity of three patients, previously reported and of 0.25 mcg. in another maturation occurred only at the site of injection without other hematologic response. In two patients, however instillation of 1 mcg. was followed by a systemic response, and generalized marrow maturation, although the latter was more advanced at the site of instillation.

"These findings suggest that in the patients who showed local maturation only the vitamin was completely bound at the injection site, whereas in those having a systemic response, only part was bound locally the remainder being available for systemic utilization. Local binding capacity is thought to be related to the degree of tissue vitamin deficiency and varies in different patients. That the effective binding substance and gastric intrinsic factor are the same has been suggested but not proven. If combination with a binding substance is necessary before vitamin B₁₂ becomes active, a deficiency of tissue binding substance might be the basic defect in certain refractory macrocytic anemias associated with megaloblastic or abnormal normoblastic bone marrow states as have been described in cirrhosis and certain other conditions."

Cleveland, Ohio

268. REISNER, E. H., JR., and KORSON R.: Microspectrophotometric determination of deoxyribonucleic acid in megakaryoblasts of pernicious anemia, *Blood* 6: 844-849 April 1951.

Authors summary "In 9 patients with various types of megaloblastic anemia responding to treatment with vitamin B₁₂, folic acid or liver extract, no significant deviations from the normal amounts of total or polymerized DNA were observed in the nuclei of red blood cells in marrow smears.

"During the maturation of megakaryoblasts in the bone marrow there is a gradual loss of nuclear DNA.

"This pattern is quantitatively and qualitatively similar for normal marrow and for that of pernicious anemia in relapse and after treatment."

New York University Post Graduate Medical School,
Bellevue Hospital, and
Columbia University
New York, N. Y.

269. DAVIDSON J. N., LESLIE, I., and WHITE, J. C.: The nucleic-acid content of the cell, *Lancet* 1 1287 1290, June 16 1951.

In experiments on the deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content of cells, the authors studied the changes in these substances in normal cells and during the course of various hematologic conditions. These acids were determined as the phosphorus compounds (D.M.A.P. and R.N.A.P.)

One of the conditions studied was megaloblastic anemia. These anemias had the greatest and most consistent deviations from normal in the amounts of cellular nucleic acids. Individual variations were generally greater with more severe anemia. In 15 observations on 8 megaloblastic anemia patients treated with liver or vitamin B₁₂, the mean amount of D.M.A.P. per cell did not change significantly during the hemopoietic response.

amount of R.N.A.P. declined rapidly toward normal, although the mean remained significantly high. The authors state "The decline in amount of R.N.A.P. was strikingly related to the return of the marrow to normoblastic erythropoiesis and to the improvement in the peripheral blood picture."

The authors comment that one advantage of knowing the D.N.A. content of the nucleus is that a determination of D.N.A. as well as other chemical constituents in a tissue sample makes possible calculation of the amount of such constituents in each cell. It is thus possible to express the composition of a tissue in terms of the average composition per cell. This method is of particular interest in considering megaloblastic anemia, in which the D.N.A. content of the nucleus is significantly high, and the greatly increased R.N.A. content of the cell explains the pronounced basophilia of the cytoplasm of younger megaloblasts.

University of Glasgow
Glasgow, Scotland
Postgraduate Medical School of London
London, England

270. MENTEN M. L., and WILLMS M.: *Nucleic acid in cells of bone marrow of patients with pernicious anemia: changes in content before and after specific treatment*, A.M.A. Arch. Path. 54 351-355, Oct. 1952.

The effect of specific therapy of pernicious anemia with vitamin B₁₂ or liver extract on the nucleic acid content of bone marrow cells was studied in 11 patients. Bone marrow aspirations were made before and after therapy was begun. The results are described as follows: "The deoxyribonucleic acid phosphorus content per cell of bone marrow aspirated from patients with pernicious anemia ranged from 1.80 to 0.50 mg. $\times 10^{-9}$. The ribonucleic acid phosphorus varied from 1.24 to 0.30 mg. $\times 10^{-9}$. The higher values were obtained in patients with anemia and symptoms of moderate severity; the lower values were found in patients in remission or with inadequate treatment over a variable time. Specific treatment caused in the bone marrow a rapid disappearance of megaloblasts with an increase in polychromatic erythroblasts and a decline in cellular nucleic acid lasting from 3 to 30 days. The duration of the decline was proportional to the degree of reduction in the initial values of cellular nucleic acid, percentile rise in polychromatic erythroblasts, and the height of the response of reticulocytes." The addition of folic acid or ascorbic acid in 2 of the patients to the B₁₂ regimen did not hasten or improve the reparative effects of vitamin B₁₂.

British Columbia Medical Research Institute, and
Framingham General Hospital
Framingham, N. C., Canada

METABOLIC PROCESSES

271. KREVANS, J. R., CONLEY C. L., and BARROWS, C. R.: *Observations on the absorption and excretion of vitamin B₁₂*, Bull. Johns Hopkins Hosp. 88 568-569 June 1951 (In Soc. Proc.)

"Crystalline vitamin B₁₂ was given parenterally to normal individuals and to patients with treated and untreated pernicious anemia. Urine collected following injection was assayed microbiologically for B₁₂ activity

Following the intramuscular injection of 25 micrograms or less of vitamin B₁₂ there was no measurable increase in the B₁₂ activity of the urine. When 100 micrograms were injected intramuscularly the B₁₂ activity of the urine collected in the first 8 hours accounted for approximately 40 per cent of the injected vitamin. There was little additional excretion of vitamin B₁₂ after the first 8 hours. When 500 micrograms or more of B₁₂ was given parenterally approximately 100 per cent of the injected vitamin could be accounted for in the urine in the first 8 hours. There was no measurable difference in the urinary excretion of vitamin B₁₂ of normal individuals and of patients with pernicious anemia. The administration of single oral doses of vitamin B₁₂ in amounts as large as 10,000 micrograms was not followed by a measurable increase in the B₁₂ activity of the urine in normal individuals or in patients with pernicious anemia. Nevertheless 4 patients with pernicious anemia in remission showed striking hematologic responses when given single oral amounts of vitamin B₁₂. Patients receiving 8000 or more micrograms of B₁₂ had complete hematologic remissions without further therapy. The absorption and excretion of vitamin B₁₂ was studied further using B₁₂ containing radioactive Cobalt 60. Five micrograms of the labelled vitamin were administered orally to a normal subject. Feces excreted in the following 4 days was homogenized, asbed, extracted with α -nitro- β -naphthol, and electroplated on copper discs from a saturated solution of ammonium oxalate. The radioactive Cobalt recovered accounted for 4.8 of the 5 micrograms administered. These observations indicate that vitamin B₁₂ is poorly absorbed from the normal gastro-intestinal tract."

The authors had expected to find that patients with pernicious anemia would not absorb orally administered vitamin B₁₂ and therefore would not excrete it in the urine. This, however was not the case. Had it been so, determining the urinary excretion of orally administered vitamin B₁₂ might have been usable as a diagnostic test in patients "with pernicious anemia who had no anemia at the time of examination." The authors plan to administer radioactive vitamin B₁₂ intravenously to patients in the near future.

Johns Hopkins Hospital
Baltimore, Md.

272. CONLEY, C. L., KREVANS J. R., CHOW B. F., BARROWS C., and LANG, C. A.: *Observations on the absorption, utilization, and excretion of vitamin B₁₂*, J. Lab. & Clin. Med. 38 84-94, July 1951.

Authors summary "Parenteral administration of as little as 25 micrograms of vitamin B₁₂ to patients with pernicious anemia in severe relapse may be followed by complete hematologic remission. When vitamin B₁₂ is administered orally in a single dose without gastric juice, approximately 5,000 micrograms are required to produce the same effect.

"Following parenteral administration of vitamin B₁₂ in amounts larger than 25 micrograms, there was prompt urinary excretion of the vitamin. When 500 micrograms or more were injected, the urinary excretion of B₁₂ was approximately quantitative. No difference was detected between the B₁₂ excretion of normal individuals and of patients with pernicious anemia.

"Oral administration of single amounts of vitamin B₁₂ as large as 10,000 micrograms was not followed by a measurable increase in the B₁₂ content of the urine of normal individuals or of patients with pernicious anemia."

The Johns Hopkins University and Hospital
Baltimore, Md.

273. NOLLIN D. L., and ROSS G. I. M.: *The vitamin B₁₂ concentrations of serum and urine of normals and of patients with megaloblastic anemias and other disease*, J. Clin. Path. 5: 129-139 1952 (abstr. Am. J. Clin. Path. 23: 382, April 1953)

"Vitamin B₁₂ microbiologically determined, was found to be present in combined and free forms. Total values of 100 to 720 µg. (mean 358 µg.) per ml. of serum were found in normal subjects, and combined values of 100 to 620 µg. (mean 329 µg.) Patients with megaloblastic anemia in the main showed lower than normal values, but patients with other diseases showed normal B₁₂ values."

274. HAUSMANN K., and MULLI, K.: *Study of metabolism of folic acid and of citrovorum factor* Acta haematol. 7: 1 Jan. 1952 (abstr. J.A.M.A. 149: 90, May 3, 1952)

"Citrovorum factor was given a therapeutic trial in 10 patients with pernicious anemia, 2 with symptomatic megaloblastic anemia, 3 with agranulocytosis, and 2 with idiopathic aplastic anemia. Two patients with pernicious anemia were given 6 mg. of synthetic citrovorum factor daily by mouth, but did not respond, and one who was given 9 mg. daily by mouth obtained only a suboptimal hematopoietic effect. In nine patients who were given 5 to 15 mg. of synthetic citrovorum factor daily intramuscularly the erythrocyte level was restored to nearly normal and the megaloblasts in the bone marrow disappeared. One patient was given a single massive intramuscular injection of 40 mg. of citrovorum factor obtained from bovine liver without significant effect on the hematopoiesis. One of the two patients with symptomatic megaloblastic anemia did not respond in the synthetic citrovorum factor while the number of erythrocytes was restored nearly to normal in the other. The patients with aplastic anemia and agranulocytosis did not respond to the treatment. Microbiological assays of the urine following administration of folic acid and citrovorum factor directly into the colon showed little evidence of absorption from this organ, but following oral or parenteral administration the urinary output increased significantly. Folic acid in high doses was found to inhibit the growth-promoting effect of vitamin B₁₂ on L. Leichmannii; high doses of citrovorum factor thymine and thymidine did not exert such an antagonistic effect. Studies of the gastric juices of patients with pernicious anemia revealed reduced catalase and increased peroxidase activity by an oxidative process the citrovorum factor is transformed to folic acid or to a substance similar in folic acid, while vitamin B₁₂ and vitamin C are destroyed. These results may explain the pathogenesis of neurological symptoms and the favorable effect of anti-biotics on pernicious anemia."

275. GIRDWOOD R. H.: *Some aspects of the metabolism of anti-megaloblastic substances in man*, Blood 8: 469-485, May 1953.

Author's summary "1 When the sera of pernicious anemia patients or controls were heated at 100 C. for 30 minutes, they developed the ability to support the growth of L. leichmannii by virtue of some substance other than vitamin B₁₂. It seemed likely however that following the administration of the vitamin, such heating also liberated free B₁₂ in the serum from a combined form.

"2. The L. leichmannii assay did not appear to be satisfactory for showing possible difference in the levels of vitamin B₁₂ in the urines or sera of pernicious anemia patients and controls.

"3. Parenterally administered vitamin B₁₂ did not cause any measurable rise in the serum level of folic acid or citrovorum factor in pernicious anemia patients or controls.

"4. The synthetic folic acid conjugates pteroyldiglutamic acid and pteroyltri glutamic acid appeared in the sera and urines of pernicious anemia patients as pteroyl glutamic acid or some related substance with folic acid activity for S. faecalis.

"5. Orally administered citrovorum factor appeared to be largely converted by the gastric juice to folic acid if free hydrochloric acid was present. When administered parenterally however citrovorum factor was excreted in the urine largely unchanged."

University of Edinburgh
Edinburgh, Scotland

276. UNGLAUB, W. G., ROSENTHAL, H. L., and GOLDSMITH, G. A.: *Vitamin B₁₂ in blood and urine after oral and parenteral administration*, Federation Proc. 12: 432, March 1953.

"Following intramuscular administration of 10 to 100 µg. of vitamin B₁₂ to normal subjects, serum vitamin B₁₂ activity rose in one hour to maximum values which were proportional to the amounts injected. Urinary vitamin B₁₂ activity in 24 hr following injection increases with the quantity administered, but cannot be predicted accurately. Serum vitamin B₁₂ activity during fasting is consistently lower in patients with untreated macrocytic anemia than in normal subjects. In 2 patients with pernicious anemia in relapse who received 25 µg. of vitamin B₁₂ intramuscularly increases in serum activity did not differ from those of normal subjects but vitamin B₁₂ activity in urine was somewhat less than normal. Serum vitamin B₁₂ activity did not increase significantly when normal subjects were given 500 and 1000 µg. orally. After oral administration of 8000 µg., serum activity reached maximum values equal to those found after intramuscular injection of 10-25 µg. Serum activity remained elevated for longer periods after oral than after intramuscular administration. In 3 of 4 patients with macrocytic anemia who received 3000 µg. of vitamin B₁₂ orally serum activity increased to maximum value similar to those noted after intramuscular injection of 1-50 µg. In the 4th no significant rise occurred. In a 5th patient serum activity increased after oral administration of 500 and 1000 µg. Optimal hematologic response resulted in all patients. Following oral doses, no significant increase in vitamin B₁₂ activity was found in the urine."

Vanderbilt University School of Medicine
New Orleans, La.

- 277 MEYER, L. M. Oral administration of Co^{57} with B_{12} in pernicious anemia. *Proc. Soc. Exper. Biol. & Med.* 82 490-491, March 1953.

Author's conclusions: "Following oral administration of 1.0 μg . of Co^{57} vit. B_{12} 1. Seven normal persons excreted 12-59% of radioactive cobalt in the stool in 3-9 days. 2. No activity could be found in the urine of 3-9 normal subjects up to 8 days after ingestion of the vitamin. 3. Four patients with pernicious anemia in remission excreted 69-99% of the dose in the stool. 4. In 3 of these cases 2 mg. of folic acid, 10 mg. of folic acid each week, 50 g. of ventriculin, and 15 mg. of intrinsic factor had no appreciable effect on the amount excreted. 5. In one case 2 mg. of folic acid and 15 mg. of intrinsic factor reduced the excretion of Co^{57} about 40% and 50 g. of ventriculin cut the fecal activity about 25%. 6. In 3 patients 2 g. of aureomycin for one week had no effect and 150 cc. of neutralized normal gastric juice reduced the fecal activity about 30%."

Columbia Memorial Hospital
New York, N. Y.
Columbia Memorial
Hospital, N. Y.

DIARRHEA

278. BARNARD R. D., and WEITZNER, H. A. B_{12} diarrhea. *Lancet* 2 717 Oct. 15, 1949 (In Letters to the Editor)

Diarrhea has been noted to attend the administration of liver extract or crystalline vitamin B_{12} to patients with Addisonian anemia. That this might be explained by a direct diuretic effect of B_{12} was suggested by the prompt disappearance of edema following injection of 15 mg. of the vitamin in an ankyrotrophic patient who had developed water retention while on adrenal cortex therapy. To test the point, 16 patients with essentially normal blood pictures were given 45 to 75 mg. of vitamin B_{12} in a single injection. During the ensuing 24 hours, 12 responded with an unequal increase in urinary output. These 12 included 1 patient with ankyrotrophic siderosis, 3 patients with chronic glomerulonephritis, 4 with Addisonian anemia in remission, 1 patient with cardiac decompensation, and 3 normal volunteers. The 4 subjects showing no definite urinary response included 2 Le Goff's earlier observations on the diuretic effect of cobalt seem to offer a more satisfactory explanation for the diuresis following B_{12} administration than the usual explanation offered, that it, changes in hematocritology. The possible clinical utility of vitamin B_{12} as a diuretic is suggested.

New York, N. Y.

- 279 JALILI, M. A. *Vitamin B₁₂ diarrhea*, *Lancet* 1 977 May 20, 1950 (In Letters to the Editor)

The writer reports 4 cases treated with single injections of 40 to 60 mg. of vitamin B_{12} . In no case was any diarrhea observed. This is contrary to the findings of Barnard and Weitzner (*Lancet* 2: 717 1949 [After single injections of 45 to 75 mg. of vitamin B_{12}].

Royal Free Hospital
London, England

280. REDFORD P. D. *Diuretic effect of vitamin B₁₂*, *Lancet* 1 1232-1233, June 2, 1951 (In Letters to the Editor)

The author describes his experiment for determining the diuretic effect of vitamin B_{12} in 87 patients with normal blood pictures. Vitamin B_{12} was given in single intramuscular 100 mg. doses to patients on diets containing not more than 650 mg. of sodium per day and permitting free intake of fluids. In no case was there a significant diuresis (defined as output exceeding intake) after vitamin B_{12} . Mercurial diuretics caused diuresis in each case although occasionally this was within the normal range for the patient.

Conley and Hospital
Oxford, England

EXCRETION OF VITAMIN B_{12}

281. CONLEY C. L., LANG C. A., CHOW B. F., and ELLICOTT C. E.: *B₁₂ activity of the urine of normal subjects and of patients with pernicious anemia following oral and parenteral administration of the vitamin*, *J. Clin. Investigation* 29 806 June 1950.

282. CHOW B. F., LANG, C. A., DAVIS, R., CONLEY C. L., and ELLICOTT C. E.: *The appearance of vitamin B₁₂ activity in urine after oral and intramuscular administration to man*, *Bull. Johns Hopkins Hosp.* 87 156-163, Aug. 1950.

Vitamin B_{12} was given intramuscularly and orally to normal subjects and to untreated patients with pernicious anemia, after which the vitamin B_{12} activity of the urine was determined by microbiological assay.

Two normal subjects were given 100 mg. of crystalline vitamin B_{12} intramuscularly. Urine collected prior to the injections showed no appreciable B_{12} activity. In the first 12 hours following the injection, the B_{12} activity of the urine was equivalent to the activity of 50 per cent of the injected material. Three patients with untreated pernicious anemia were given 100 mg. of crystalline vitamin B_{12} intramuscularly. Most of the vitamin administered appeared in the urine.

In two normal subjects given 100 mg. of crystalline vitamin B_{12} orally urine collected over a subsequent 48-hour period showed no detectable B_{12} activity.

Three normal subjects were given a larger dose (500 mg.) of vitamin B_{12} intramuscularly and 3 others received 500 mg. of the vitamin orally. In the 3 subjects receiving the oral dose, the vitamin did not appear in the urine. However B_{12} activity promptly appeared in the urine of the 3 subjects receiving the vitamin parenterally. The B_{12} activity of the urine diminished rapidly about eight hours after the injection.

The failure of B_{12} activity to appear in the urine of normal subjects after oral administration of the vitamin may indicate that it is poorly absorbed from the gastrointestinal tract even by normal individuals. The authors had anticipated that vitamin B_{12} activity would appear in the urine of normal subjects after oral administration but that the activity would not appear when the vitamin was given orally to patients with pernicious anemia.

It is conceivable that vitamin B₁₂ is altered in some way during its passage through the gastrointestinal tract so that it is no longer capable of being excreted by the kidneys, or that it might undergo some change during its absorption which would render it ineffective in meeting the nutritive requirements of the microorganism employed in the assay.

The concomitant administration, by either the oral or parenteral route of biotin and vitamin B₁₂ was followed by the appearance of free biotin in the urine.

John F. Ford is University
Instructor, M.D.

283. SOKOLOFF M. F., SANNEMAN E. H., Jr., and BEARD M. F. *Urinary excretion of vitamin B₁₂*. Blood 7 213-250, Feb. 1952.

The urinary excretion of vitamin B₁₂ was studied in 12 patients, 6 of whom had pernicious anemia. Dosages were 42.2, 63.3, 84.4, 211.0 mcg. given parenterally both in ascending and descending order at daily intervals. The two larger amounts resulted invariably in the excretion of 53 to 63 per cent of the injected vitamin B₁₂ within 18 hours. The percentage of excretion rose remarkably as the dosage increased. When 42.2 mcg. was given, there was relatively little excretion when this was the initial dose, but the excretion was appreciable when these doses followed the injection of the larger doses. "Saturation" of the body apparently occurs. In general more vitamin B₁₂ is excreted in the smaller doses if these follow the larger doses. No significant differences could be detected between the pernicious anemia and non-pernicious anemia patients.

University of Louisville School of Medicine
Louisville, Ky.

284. CUTHBERTSON, W. F. J. *Clinical and laboratory aspects of vitamin B₁₂*. Lancet 2 133-134, July 19 1952 (in Soc. Proc.) also Brit. M. J 2 153-154 July 19 1952 (in Soc. Proc.)

In a study of the excretion of vitamin B₁₂, it was found that maximal urinary output occurred some four hours after intramuscular injection of 200 mcg. little was excreted after seven hours. With injection of 1,000 mcg., 50 per cent of the dose was eliminated by a normal person and about 60 per cent by patients with pernicious anemia. Less of the vitamin was excreted when the anemia was in remission than during relapse. Following doses of 3,000 to 9,000 mcg. orally excretion was much less than expected. Possibly the vitamin was taken up by the intestinal mucosa into the abdominal lymphatics, or in the liver. Lymphatic absorption seemed unlikely because the excretion following intramuscular injections at laparotomy was much the same as that after intramuscular injection.

285. ROSS G. I. M. *Clinical and laboratory aspects of vitamin B₁₂*. Brit. M. J 2 154, July 19 1952 (in Soc. Proc.) Lancet 2 134, July 19 1952 (in Soc. Proc.)

"Dr G. I. M. Ross (London) reported on assays depending on the use of *Escherichia gracilis* which was sensitive to 1 µg./ml. In patients with megaloblastic anemia due to lack of vitamin B₁₂ the level of B₁₂ in the serum was nearly always low while if the megaloblastosis was

due to folic acid lack, the B₁₂ levels were normal. In about 65% of B₁₂ deficient subjects a single estimate might give falsely high values, but repeated tests gave lower figures. When injected, vitamin B₁₂ combined with serum protein in the course of eight hours, and during this period some of the uncombined B₁₂ was excreted thereafter a constant level was reached which persisted for some days. The concentration of B₁₂ in the serum and the duration of the rise depended mainly on the dose administered but also on the subject. Saturation occurred after injections given daily for 7 to 10 days."

286. MONTO R. W. *Respiratory tract absorption of crystalline B₁₂ in man demonstrated by urinary bioassay and hematopoietic studies* Federation Proc. 12 396, March 1953.

"After oral administration of B₁₂ at dosages as high as 10,000 mcgm., B₁₂ growth activity in the urine has been shown to be less than 0.05 millimicrograms/ml. Upon inhalation of 200 mcgm. of B₁₂ in saline, B₁₂ activity in the urine reached the level of 0.26 millimicrograms/ml. and following inhalation of approximately 500 mcgm. of B₁₂ in lactose powder B₁₂ activity in the urine rose to 0.134 millimicrograms/ml. Direct pulmonary instillation of 100 µg. of crystalline vitamin B₁₂ by means of the bronchoscope, resulted in a urinary excretion (9.76 millimicrograms/ml.) pattern equivalent to the intramuscular injection of 60-80 mcgm. of B₁₂. Inhalation therapy with crystalline B₁₂ in physiological saline and lactose dust has resulted in adequate clinical and hematological response in five patients with pernicious anemia in relapse. A sixth patient demonstrated maximal response to a single intranasal application of nose drops of 100 µgm. crystalline B₁₂ in 1 ml. of saline. Twenty five pernicious anemia patients in remission have been satisfactorily maintained by inhalation treatment for periods up to 1 year."

Henry Ford Hospital
Detroit, Mich.

CHEMISTRY

287. ABBOTT L. D., JR., and JAMES G. W., III. *Effect of vitamin B₁₂ on the urinary phenol fractions in pernicious anemia*, J. Lab. & Clin. Med. 35 35-42, Jan. 1950.

Authors summary: "The effect of crystalline vitamin B₁₂ on the excretion of tyrosine metabolites in pernicious anemia was studied by fractionating the ether soluble urinary phenols into a fraction containing the hydroxyphenyl acids (A) and a fraction containing ether soluble phenols not soluble in sodium bicarbonate (B). Three of the four patients studied were noted to have elevated pretreatment phenol fraction ratios (A:B) which decreased after the parenteral administration of crystalline vitamin B₁₂. In two of these patients the decrease in ratios was noted to precede the reticulocyte response. The one patient who did not have elevated pretreatment ratios was a Negro man who responded to vitamin B₁₂ therapy with an increase in ratios which preceded reticulocytosis. Crystalline vitamin B₁₂ in the very small amounts needed to produce remission in pernicious anemia was found to have a prompt effect on tyrosine metabolism as indicated by these variations in the excretion of tyrosine metabolites."

Medical College of Virginia
Richmond, Va.

288. JAMES G. W., III, and ABBOTT L. D., JR. *Studies on nitrogen and phosphorus metabolism with relation to the hematologic response in pernicious anemia patients after crystalline vitamin B₁₂*. *Am. J. Med.* 11: 521, Oct. 1951 (in Soc. Proc.)

"In view of the response in hemoglobin synthesis caused by crystalline vitamin B₁₂ in patients with pernicious anemia it was considered desirable to correlate hematologic observations with nitrogen and phosphorus balance studies before and during clinical remission in an attempt to elucidate further the metabolic effects of this substance. Four previously untreated patients with pernicious anemia were studied for several weeks on a constant diet. Positive nitrogen balance as great as 6 gm. per day was produced by vitamin B₁₂ in each case. When negative nitrogen balance existed during the control period, it promptly became positive. Individual patients made from 18 to 37 gm. of hemoglobin per day for an average period of eleven days. Nitrogen required for hemoglobin formation alone equalled or exceeded the total nitrogen retained during the corresponding period.

"The following sequence of biochemical changes was observed. First, a prompt and striking decrease in urinary phosphorus which preceded any change in reticulocyte count; second, increased excretion of uric acid during the appearing of reticulocytes and third, increased excretion of phosphorus during the period of greatest reticulocytosis. These clinical observations suggest that among the first metabolic effects of vitamin B₁₂ is a profound influence on nucleoprotein synthesis."

Richmond, Va.

289. JAMES, G. W., and ABBOTT L. D., JR.: *Metabolic studies in pernicious anemia. I. Nitrogen and phosphorus metabolism during vitamin B₁₂-induced remission*, *Metabolism* 1: 259-270, May 1952.

Authors summary of their observations on 4 patients

"1. Positive nitrogen balance was found to exist, as much as 6.0 gm. per day during remission induced by vitamin B₁₂ in pernicious anemia. Nitrogen accumulated as blood proteins exceeded the dietary intake of nitrogen, indicating conversion of tissue nitrogen to blood proteins.

"2. Urinary phosphorus followed a pattern of rapid diminution after therapy then an increased excretion during reticulocytosis, and a gradual return to a normal excretion. This was in part responsible for a positive phosphorus balance immediately after therapy a negative balance during reticulocytosis, and then a positive phosphorus balance.

"3. The decrease in the urine phosphorus excretion is one of the earliest biochemical alterations we have observed in therapy induced remission and is probably related to a fundamental effect of vitamin B₁₂ on nucleoprotein metabolism occurring in the change from a megaloblastic to an erythronormoblastic marrow

"4. Uric acid excretion increased with reticulocytosis and is considered a reflection of accelerated nucleoprotein metabolism."

Medical College of Virginia
Richmond, Va.

FECAL CONTENT OF VITAMIN B₁₂

290. CALLENDER, S. T. E., MALLETT B. J., SPRAY, G. H., and SHAW G. E. *Anti-anemia activity of fecal extract from pernicious anemia patient preliminary communication*, *Lancet* 2: 57 July 9 1949

A fecal extract was obtained from a patient with pernicious anemia, which on assay with *Lactobacillus lactis* Dornier had activity equivalent to 1 mcg. of vitamin B₁₂ per cc. It produced an optimal reticulocyte response and a rise in hemoglobin in a patient with untreated pernicious anemia when it was administered intramuscularly in a daily dosage of 5 cc. for five days. Also, the marrow of the patient, which previously had been megaloblastic, became definitely normoblastic, and subjective symptoms of subacute combined degeneration of the central nervous system improved. A chromatogram of the extract closely resembled chromatograms of purified parenteral liver extracts, which suggests that the anti-pernicious anemia activity of the fecal extract was due to vitamin B₁₂.

291. GIRDWOOD R. H.: *The intestinal content in pernicious anemia of factors for the growth of Streptococcus faecalis and Lactobacillus leichmannii*, *Blood* 5: 1009-1016, Nov. 1950.

During a study of 3 cases of untreated pernicious anemia and 2 control patients, it was found that the gastric juices and small intestinal secretions obtained during a period of fasting contained only very small amounts of folic acid or the growth factors for *Lactobacillus leichmannii*, which include vitamin B₁₂. No evidence was found of a consistent increase or decrease of these factors along the intestine.

Both folic acid and vitamin B₁₂ appear to be synthesized in relatively large amounts in the large intestine. The daily output in the stools may approximate 5 mcg. of vitamin B₁₂ and 0.5 mg. of folic acid in patients with pernicious anemia as well as in control patients.

The author concludes from his results that folic acid and vitamin B₁₂ are produced by bacteria in the large intestine of man, apparently at least as well in pernicious anemia patients as in normal subjects. It is thought unlikely that relapses and spontaneous remissions in pernicious anemia can be associated with changes in the intestinal flora of the small intestine, leading to the destruction or formation of significant amounts of folic acid or vitamin B₁₂ at the levels of maximum absorption. However the author states that the data presented do not entirely rule out such a mechanism.

In an addendum is reported the rectal administration to a pernicious anemia patient of 100 cc. of normal gastric juice together with 25 mcg. of vitamin B₁₂ daily for four days. No beneficial effect was observed. The administration of 100 cc. of normal gastric juice by mouth together with 25 mcg. of vitamin B₁₂ daily for four days produced a reticulocytosis of 8.4 per cent and a rise of red cells from 2,640,000 to 3,170,000 per cu. mm. over a period of 20 days.

Thomas Henry Simpson Memorial Institute for Medical Research
Ann Arbor, Mich.

292. CALLENDER S T F., and SPRAY G H: *Preparation of haemopoietically active extracts from jaccet*, *Lancet* 1 1391-1392, June 30 1951

In experimental studies extracts were prepared from the feces of a normal person and from 2 patients with untreated pernicious anemia. This material produced improvement when given by intramuscular injection to patients with Addisonian anemia in relapse. The authors state that the active substance in the extracts appears to be B₁₂, and seem to be similar in amount in a normal person and in test patients with untreated pernicious anemia.

British Medical Journal
Oxford, England

CLINICAL TEST FOR TOXIC REACTION

293. BEDFORD P D: *Side-effects of a preparation of vitamin B₁₂*, *Brit. M. J.* 1 690-691 March 29 1952.

In an effort to determine whether vitamin B₁₂ itself or a contaminant was responsible for side effects noted, three vitamin B₁₂ preparations were tested on 100 people.

None reacted adversely to a solution of vitamin B₁₂ derived from liver and containing 10 mcg. of the vitamin per cc.

Fourteen people were skin-test positive to a proprietary preparation containing B₁₂ 20 mcg./cc. and derived by customary extraction procedures from *Streptomyces fermentation liquors*. Six suffered side effects when this solution was given intramuscularly.

Two people were skin-test positive to another similar proprietary preparation except that it was "purified by crystallization and therefore likely to contain a smaller proportion of any impurity." None were sensitive to its intramuscular use. Impurities carried over from the mold fermentation liquor were regarded as responsible for the sensitivity phenomena. Both cutaneous reactions and side effects were found to be more than twice as common in people who had previously been treated with antibiotics. The author advanced the possibility of inducing idiosyncrasy to antibiotics as a reason against the use of impure preparations of vitamin B₁₂ derived from mold cultures.

Country Food Research
Oxford, England

ANIMAL STUDIES

TOXICITY STUDIES

294. WINTER, C. A., and MUSHETT C. W: *Absence of toxic effects from single injections of crystalline vitamin B₁₂*, *J. Am. Pharm. A. (Sc. Ed.)* 59 860-361, June 1950.

The authors administered crystalline vitamin B₁₂ to mice in doses of 100, 200, 400 and 1600 mg. per kg. intraperitoneally and in doses of 800 and 1600 mg. per kg. intravenously. In addition, the vitamin was injected intraperitoneally in rats and guinea pigs in doses up to 100 mg. per kg. In no instances were there any deaths or toxic manifestations. Some of the mice were sacrificed 48 hours or one week after the injection was given, and no gross pathologic changes were observed at autopsy. These results differ from those of Trause (Arch. Path. 49 278, 1950 [Abstr. 295]) who described toxic reactions and deaths in mice treated with a concentrate containing vitamin B₁₂; intraperitoneal or subcutaneous injections of 8 mg. per kg. of B₁₂ in this form produced death in all of the treated animals. Since the largest doses of the crystalline vitamin used by the present authors was more than 500 times as great as those of the B₁₂ concentrate used by Trause, it appears that the toxic manifestations observed by him were probably due either to impurities in the preparation, or to contamination of the sample. On the basis of the present results crystalline vitamin B₁₂ is essentially a nontoxic substance.

Food Institute for Therapeutic Research
Baltimore, Md.

295. TRAINE, V: *Toxicity studies on vitamin B₁₂ in albino mice*, *Arch. Path.* 49 278-279 March 1950.

It was found that in the albino mouse an intraperitoneal dose of about 0.75 mg./Kg. of the vitamin B₁₂ preparation used is not toxic; an intraperitoneal dose

of 1.5 mg./Kg. is decidedly toxic; and a dose of 3 mg./Kg., given either intraperitoneally or subcutaneously is lethal for 100 per cent of the animals treated. Congestion and edema of the lungs and slight congestion of the visceral organs were the only pathologic changes observed in the animals that died.

In a separate study it was found that guinea pigs showed no sensitivity to vitamin B₁₂. The author cites unpublished data of his own which show that vitamin B₁₂ possesses antianaphylactic and antihistaminic activity.

Western Park Hospital
Cleveland, Ohio

ANEMIAS; OTHER BLOOD STUDIES

Chicks

296. WAGLEY P F., and MORGAN, H. R: *Observations on the effects of folic acid antagonists, folic acid, liver extract and vitamin B₁₂ on embryonated eggs: a preliminary report*, *Bull. Johns Hopkins Hosp.* 83: 275-278, Sept. 1948.

Chick embryos were inoculated via the yolk sac with three folic acid antagonists, alone, or 24 hours after folic acid, liver extract, or vitamin B₁₂ had been introduced into the sac. 4-Amino-pteroylglutamic acid in amounts of 0.005 mg. decreased the survival time of the embryos and produced cytologic changes in the yolk-sac blood islets (diminution in the size and number with karyolysis and karyorrhexis of many of the remaining nuclei). This effect was not altered by the injection of 0.005 mg. of vitamin B₁₂ simultaneously with the inhibitor or by the injection of 3.75 U.S.P. units of purified liver extract (with activity equivalent to that of 2.7 micrograms of vitamin B₁₂). However when 12.5 mg. of folic acid was injected 24 hours prior to the antagonist the blood islets

were not decreased in number although their cellularity was somewhat diminished. Pyknosis, karyolysis and karyorrhexis of the nuclei were not present. There was a suggestive increase in survival time.

The changes produced by methyl-4-amino-pteroyl glutamic acid, which were similar to those produced by 4-amino-pteroylglutamic acid but less marked, were altered by folic acid but not by liver extract.

N¹⁰-methyl-pterole acid caused no detectable changes in the yolk sac blood islets.

- 297 WAGLEY P F., and MORGAN, H. R. *Effects of folic acid antagonists inoculated in embryonated eggs*, Arch. Path. 46 441-450, Nov 1948.

Chick embryos were inoculated via the yolk sac with three folic acid antagonists, alone or 24 hours after folic acid, liver extract, or vitamin B₁₂ had been introduced into the sac. 4-Amino-pteroylglutamic acid caused pronounced changes in the hemopoietic tissue of the embryos, decrease in the number, size, and cellularity of the blood islets of the yolk sac and pyknosis and nuclear fragmentation of the cells that remained. Methyl-4-amino-pteroyl glutamic acid in comparable doses had much less effect on the blood islets, and N¹⁰-methylpterole acid in relatively large doses had no appreciable effect. The effects of 4-amino-pteroylglutamic acid were counteracted by folic acid, but unaltered by liver extract or by vitamin B₁₂. The survival time of embryos inoculated with 4-amino-pteroylglutamic acid decreased with increasing dosage of the antagonist. The technique employed in this study are described. The yolk sac method is a new way of evaluating the effects of folic acid inhibitors.

*Reprints: Cary H. Campbell, and
Edward Nelson Nelson
Baltimore, Md.*

298. NICHOL, C. A., HARPER, A. E., and ELVEH JEM, C. A. *Effect of folic acid, liver extract, and vitamin B₁₂ on hemoglobin regeneration in chicks* Proc. Soc. Exper. Biol. & Med. 71: 34-37 May 1949 Federation Proc: 8 233, March 1949

Severe anemia was induced in chicks by the intramuscular injection of phenylhydrazine hydrochloride following a depletion period on a folic acid-deficient diet. The effect of folic acid, liver extract, and vitamin B₁₂ on the rate of hemoglobin regeneration in these anemic chicks was studied.

Liver extract alone did not influence the rate of hemoglobin formation. The combination of liver extract and folic acid caused a more rapid regeneration of hemoglobin than could be obtained with folic acid alone. Vitamin B₁₂ completely replaced liver extract in stimulating the formation of hemoglobin in the presence of folic acid.

Thus it appears that the component of liver extract causing the stimulation of hemoglobin regeneration in the presence of folic acid is vitamin B₁₂. The metabolic interrelationship between folic acid and vitamin B₁₂ is not known. It may be that the vitamin makes more folic acid available to the animal. However it may also act directly in the normal mechanism of hemoglobin formation, as is implied by its activity in patients with pernicious anemia. The results of the present study indicate that vitamin B₁₂

does not have optimum activity on hemoglobin regeneration in chicks unless folic acid is present in adequate amount.

- 299 DE GUIA, E. F., and WOLFRED M. M.: *Comparative hemopoietic and growth factor responses to vitamin B₁₂, folic acid and iron*, J. Am. Pharm. A. 60 299-301 June 1951.

The authors report further investigations on the response of the hemopoietic and growth factors in anemic chicks to vitamin B₁₂, folic acid, and iron. The basal diet included methionine, 0.3 Gm. thiamine, 0.4 mg.; riboflavin, 0.8 mg. nicotinic acid, 5.0 mg.; pyridoxine, 0.6 mg.; calcium pantothenate, 2.0 mg. biotin, 0.02 mg. choline chloride, 200.0 mg.; inositol, 100.0 mg.; α-tocopherol, 0.30 mg. vitamin K₁, 2.50 mg. Chicks became anemic (red blood cells 2 million per cu. mm. and hemoglobin 5 Gm. or less per 100 cc.) in 24 to 30 days. The mortality rate before anemia was reached was 25.6 per cent. Anemic chicks showed body tremors, extended wings and poor balance, signs of paralysis, and poor growth and feathering.

Vitamin B₁₂ supplements did not cause significant improvement in red blood cell and hemoglobin levels. Parenteral administration of B₁₂ in doses of 6 mcg. produced greater growth, while chicks receiving 3 mcg. per 100 Gm. of feed died within ten days after treatment was begun. No improvement in feathering was noted.

All the chicks given daily supplements of 6 mcg. of folic acid parenterally showed rises in red cell counts and hemoglobin levels, but normalcy was not reached. These chicks showed a greater weight increase than those which received B₁₂, and normal feathering returned.

Chicks receiving 6 mcg. B₁₂ plus 6 mcg. of folic acid showed the greatest increase in weight. Blood counts and hemoglobin levels returned to normal after nine parenteral injections or after 12 days of oral administration. Normal feathering began to appear within two days.

Chicks treated with 3 mcg. ferric ammonium citrate plus 3 mcg. of B₁₂ given parenterally showed slightly greater weight increases than those treated with B₁₂ alone. However a hemopoietic response comparable to that produced by 6 mcg. of folic acid plus 6 mcg. of ferric ammonium citrate was produced by 6 mcg. of B₁₂ plus 6 mcg. ferric ammonium citrate. No improvement in feathering was noted.

Chicks treated with folic acid and iron parenterally or orally showed greater weight gains than all others and higher red cell and hemoglobin levels than those receiving folic acid alone, but did not achieve normalcy. Normal feathering developed within four days.

Thus, of all supplements tested, only the combination of folic acid and B₁₂ cured the anemia. There is evidence that B₁₂ may function somewhat as a catalyst by facilitating the uptake or release of folic acid by body tissues.

*University of Southern California
Los Angeles, Calif.*

300. STERN J R., HSU J M., and MCGINNIS, G.: *Vitamin B₁₂ and hemoglobin regeneration in the chick*, J. Biol. Chem. 194 191-196, Jan. 1952.

The effect of vitamin B₁₂ deficiency upon hemoglobin formation in the chick was studied. Four groups of from 12 to 30 chicks each, hatched from hens receiving a B₁₂-deficient ration were fed a corn-soybean oil meal basal fortified with B-vitamins, vitamins A and D and terramycin (68 mg./lb.). Two groups received 30 mcg. of crystalline B₁₂ per kg. of diet. Two mg. of phenylhydrazine hydrochloride per 100 Gm. of body weight was injected subcutaneously on alternate days into one of the B₁₂-supplemented lots and one on the basal alone. The phenylhydrazine administration was begun when the chicks were 13 days old. In a second trial the drug was given at 6 days of age.

Unlike the rat, mouse, pig, fox, and mink, the chick was able to maintain normal hematocrit and hemoglobin levels in an uncomplicated vitamin B₁₂ deficiency although the growth rate was severely retarded. When the vitamin B₁₂ deficiency was aggravated by an anemia resulting from injections of phenylhydrazine hydrochloride, vitamin B₁₂ was shown to have a stimulatory action on hemoglobin and hematocrit. In the presence of phenylhydrazine, chicks fed the B₁₂ had significantly higher hemoglobin, hematocrit, and erythrocyte concentrations than chicks deficient in B₁₂. A single injection of phenylhydrazine into 3 week old chicks caused anemia, and a seven day period elapsed before the hemoglobin level of deficient chicks reached a normal level, whereas only five days were required for hemoglobin of vitamin B₁₂-supplemented chicks to return to normal.

Terramycin, which was fed to all groups and which stimulated growth of chicks on a complete diet, did not replace B₁₂ as a growth stimulant.

Phenylhydrazine Hydrochloride
Pittsburg, Pa.

Mice

301. VIJAYARAGHAVAN P. K., and DUNN M. S.: *Effect of crystalline vitamin B₁₂ on experimental anemia in mice* Proc. Soc. Exper. Biol. & Med. 75: 754-756, Dec. 1950.

Crystalline vitamin B₁₂ injected intraperitoneally into mice with anemia experimentally induced by phenylhydrazine increased the number of red blood cells per unit volume, but not the hemoglobin concentration. Prior to and during the experiment the mice were kept on a chemically defined diet probably containing a relatively small amount of vitamin B₁₂ but adequate amounts of other hematopoietic substances. The increase in red blood cells during the first four days of severe anemia was found to be directly proportional to the amount of vitamin B₁₂ administered. These results suggest the possible development of an assay procedure for the determination of vitamin B₁₂.

University of California
Los Angeles, Calif.

302. VIJAYARAGHAVAN P. K., and DUNN, M. S.: *Effect of vitamins B₁₂, B_{12m}, and B_{12c} on red blood cell counts in experimental anemia in mice* Arch. Biochem. 31: 248-250, April 1951.

The potencies of vitamins B₁₂, B_{12m}, and B_{12c} in raising the red blood cell counts in anemic mice were

tested. Anemia was produced by a single intraperitoneal injection of phenylhydrazine hydrochloride, 6 to 8 mg. per 100 Gm. of body weight. This dosage had been found to reduce the red blood cell count to 50 per cent of its normal value. Following injection of 2.00 to 2.16 mcg. daily of several manufacturers' vitamin B₁₂ preparations for two or four days after the peak of anemia had been reached, the red blood cell count in those animals that received B₁₂ was more than twice that of the control mice. Increases averaged 0.89 millions per cu. mm. after two days of treatment, and 0.30 millions per cu. mm. after four days. Differences among the various B₁₂ preparations were not significant. The authors assume, therefore, that these B₁₂ preparations are similar if not identical, in composition and structure of the chemical groups responsible for the effects noted.

University of California
Los Angeles, Calif.
Radiation Research Laboratories
Cancer Institute

303. VIJAYARAGHAVAN P., and DUNN M.: *Effect of thymidine, citrovorum factor, folic acid and degradation products of vitamin B₁₂ on red blood counts in experimental anemia in male mice* Arch. Biochem. & Biophysics 36: 299-303, 1952 (abstr. Blood 7: 856-857 Aug. 1952).

"The hematopoietic effect of various agents was tested on male mice following the peak of anemia induced by intraperitoneal injection of phenylhydrazine hydrochloride. The red blood cell counts determined under these conditions were increased comparably by injections of the citrovorum factor at high levels (200 mcg.) and vitamin B₁₂ at low levels (2 mcg.). The compounds thymidine, alpha ribazole, 5,6-dimethylbenzimidazole, 1-D-riboyl-5,6-dimethylbenzimidazole, and 1,2-diamino-4,5-dimethylbenzene exhibited little or no antianemic activity while the synthetic analog (2,5-dimethylbenzimidazole) of these degradation products of vitamin B₁₂ inhibited red blood cell formation to a level below that obtained on a folic acid-free diet."

University of California
Los Angeles, Calif.

Rats

304. CAMERON D. G., CALLENDER, S. T. E., WATSON G. M., and WITTS, L. J.: *Experimental macrocytic anemia in the rat treated with purified liver extract, pteroylglutamic acid and vitamin B₁₂* Nature 164: 188 July 30, 1949 (Letter to Editor).

Pteroylglutamic acid prolonged the survival time of rats in which experimental macrocytic anemia had been produced by formation of a cal-de-sac in the small intestine, whereas purified liver extract and vitamin B₁₂ did not. Hematologic remissions occurred with all three substances, but more frequently with pteroylglutamic acid. One control animal had a spontaneous remission.

Biological Laboratory
Oxford, England

305. KODICEK, E., and CARPENTER, K. J.: *Experimental anemias in the rat. II The effect of various sulfonamides in producing a pteroylglutamic acid deficiency and the pteroylglutamic acid activity of test substances*, Blood 5: 540-552, June 1950.

Authors summary "Different sulfonamides were tested to ascertain their effect in producing the characteristic symptoms of acute PCA deficiency in rats fed on synthetic diets. Sulfasuxidine (1 per cent) and the less soluble phthalylsulfathiazole (1 per cent) were equally effective. Sulfathiazole in 1 per cent concentration produced a hemolytic anemia not reversible by PCA or whole liver powder. In a 0.5 per cent concentration it was also effective, but in view of its toxicity the less soluble sulfonamides were to be preferred. A mixture of 0.5 per cent sulfathiazole and 0.5 per cent sulfadiazine was extremely toxic and produced a hemolytic anemia. Sulfaguanidine was toxic at 1 per cent concentration.

"When intermittent small doses of PCA were given to PCA-deficient rats to prolong their life from 45 up to 155 days, 1 per cent sulfasuxidine or phthalylsulfathiazole, or 0.5 per cent sulfathiazole were equally efficient in producing regularly a macrocytic normochromic anemia.

"The response of PCA-deficient rats to single doses of PCA has been studied and an assay procedure has been suggested which uses the weight increase and duration of cure as the measure of the response. The W.B.C. and reticulocyte response can also be used as a qualitative indication of PCA activity

"Of the substances tested by this procedure, vitamin B₁₂, purified pernicious anemia preparations, ascorbic acid and xanthopterin showed no PCA activity. A commercial yeast preparation and Teropterin were found to possess biologic activity comparable with that found by other workers in assays on chicks."

University of Cambridge, and
Medical Research Council
Cambridge, England

306. BORSON H. J., SINGMAN D., LEFKOVSKY S., DIMICK, M. K., GASC, V., and PERRY R.: Hematologic changes and death in vitamin B₁₂-deficient rats. *Am. J. Physiol.* 162: 714-720, Sept. 1950.

A high mortality was observed within three weeks after weaning in rats weaned on vitamin B₁₂-deficient diets. Death was associated with a leukopenia and granulocytopenia which were not per se the causes of death. It is suggested that these were manifestations of a profound biochemical or anatomic lesion which was the actual cause of death. Vitamin B₁₂ deficiency possibly is associated with disturbed protein metabolism.

Some of the animals were given crystalline vitamin B₁₂ or vitamin B₁₂ concentrate in doses of 1 mcg. subcutaneously twice a week. In all except the moribund animals this treatment resulted in prompt improvement—recovery from leukopenia, granulocytopenia, anemia, and rapid and sustained weight gain. No difference was observed in the effects of the two vitamin B₁₂ preparations.

A few animals were treated with the sodium salt of folic acid (0.5 mg. subcutaneously twice a week). Results were favorable in some of these, but were not nearly so consistent or spectacular as results obtained with vitamin B₁₂.

Some of the animals recovered spontaneously. This was thought to be due, perhaps, to adaptive mechanisms,

to changes in vitamin B₁₂ requirements, or to the availability of vitamin B₁₂ from intestinal synthesis.

University of California Medical School
Berkeley, Calif.
University of California
San Francisco, Calif.

307. JØRGENSEN, R., and STUDER, A.: Experimental leukopenia in rats and their therapeutic response to vitamins and other substances, *Acta haematologica* 5: 47-64, 1951 (abstr. *Blood* 7: 382, March 1952).

"Succinylsulfathiazole was fed to 1,000 rats in order to produce a striking granulocytopenia. This was used as a test for the examination of the leukopoietic effect of various substances. Especially effective was folic acid, even in experiments with additional damage of the bone marrow by nitrogen mustard. Vitamin A and C and iron had a similar leukopoietic effect. No response was seen using the vitamins B₂, B₆, B₁₂, E, the nucleic acid derivatives hypoxanthine, deoxyribose, guanine, deoxyribose, thymine, thymidine, uracil, furthermore [sic] choline, testosteronepropionate, methionine and thyroxine."

Hoffmann-La Roche Co.
Basle, Switzerland

308. WATSON G. M., and WITTS, L. J.: Intestinal macrocytic anemia, *Brit. M. J.* 1: 13-17 Jan. 5, 1952.

Megaloblastic anemia was induced in rats by forming a blind loop in the middle third of the intestine. The anemia was associated with hemolysis and sometimes with steatorrhea. Response to folic acid or succinylsulfathiazole was good, but to vitamin B₁₂ poor or absent. With reference to the mechanism of the experiment the authors state: "From the available evidence the most likely cause of the anemia is an alteration of the intestinal flora analogous to that which occurs in cases of gastro-colic fistula in man, and it is possible that the intestinal bacteria are in some way concerned with the absorption or utilization of haemopoietic substances."

Robt. J. University
Oxford, England

309. CRAFTS, R. C.: The effects of cobalt, liver extract, and vitamin B₁₂ on the anemia induced by hypophysectomy in adult female rats, *Blood* 7: 863-873, Sept. 1952.

Authors summary: "Hypophysectomized adult female rats of the Wistar strain were treated with daily subcutaneous injections of 0.1, 0.3 and 0.5 mg. of cobalt nitrate for 50 days. Most striking results were obtained with the largest dose. The erythrocyte count in the hypophysectomized rats was elevated from a preoperative level of 7.9 million cells per cu. mm. to 11.6 million cells; the hematocrit, from 45.8 per cent to 60.8 per cent; and the hemoglobin, from 16.0 Gm. per 100 cc. to 21.0 Gm. Normal animals respond in a similar manner. Cobalt nitrate, therefore, not only prevented anemia but actually induced a marked elevation in the number of erythrocytes and in hemoglobin. Vitamin B₁₂ [1 or 2 mcg. daily subcutaneously] and liver extract were ineffective."

University of Chicago College of Medicine
Chicago, Illinois

Swine

310. HEINLE, R. W., WELCH, A. D., and PRITCHARD J. A.: *Essentiality of both the antipernicious anemia factor of liver and pteroylglutamic acid for hematopoiesis in swine*, J. Lab. & Clin. Med. 33: 1647 Dec. 1948 (In Soc. Proc.); Proc. Central Soc. Clin. Res. 21: 94 1948.

Cleveland, Ohio

311. CARTWRIGHT G. E., and WINTROBE, M. M.: *Experimental production of a nutritional macrocytic anemia in swine* Federation Proc. 8: 351-352, March 1949

A nutritional macrocytic anemia was produced in 32 swine fed a diet deficient in pteroylglutamic acid. This was not prevented by the presence of "extrinsic factor" in the diet. Purified liver extracts as well as vitamin B₁₂ possessed some hemopoietic activity under the conditions employed, but less than pteroylglutamic acid compounds.

312. CARTWRIGHT G. E., TATTING B., ASHENBRUCKER, H., and WINTROBE, M. M.: *Experimental production of a nutritional macrocytic anemia in swine* Blood 4: 301-323, April 1949

A macrocytic anemia, similar morphologically to pernicious anemia and the related macrocytic anemias in man, was induced in swine made deficient in pteroylglutamic acid by a purified diet containing casein (10 or 26%) and supplemented with B vitamins, sulfasuxidine, and a crude methyl folic acid antagonist. Administration of pteroylglutamic acid compounds caused a rapid return of both blood and bone marrow to normal. Purified liver extracts and crystalline vitamin B₁₂ showed some hemopoietic activity in several animals.

313. PALMER, J. G., CARTWRIGHT G. E., and WINTROBE, M. M.: *Experimental production of a nutritional macrocytic anemia in swine*, Am. J. Med. 8: 541-542, April 1950 (In Soc. Proc.)

A folic acid deficiency characterized by macrocytic anemia was produced in swine. This deficiency was corrected by the administration of pteroylglutamic acid and some of its derivatives, but was only partially corrected by purified liver extract, vitamin B₁₂, ascorbic acid, and other substances. The administration of thymine and vitamin B₁₂ together did not substitute for pteroylglutamic acid.

Experiments with 2,6-diaminopurine showed that it produced a metabolic deficiency differing from that produced by the "crude" methyl folic acid antagonist used in the first experiments.

Attempts were made to produce a deficiency of vitamin B₁₂ in swine by raising young pigs on soybean protein rather than on a casein diet. Pteroylglutamic acid, sulfasuxidine and, in certain instances, desiccated thyroid were included in the diet. Following the administration of crystalline vitamin B₁₂ to such animals a growth response was observed. The deficiency syndrome, however, was not accompanied by significant anemia or striking alterations in the bone marrow

University of Utah College of Medicine
Salt Lake City, Utah

314. CARTWRIGHT G. E., PALMER, J. G., TATTI B., ASHENBRUCKER, H., and WINTROBE, M. M.: *Experimental production of nutritional macrocytic anemia in swine. III Further studies on pteroylglutamic acid deficiency* J. Lab. & Clin. Med. 67:5-693, Nov 1950

In their summary the authors point out that experimental anemia in swine which is described has more similarities to refractory megaloblastic or achrestic anemia in man and in many instances of true macrocytic anemia, megaloblastic anemia of infancy pernicious anemia of pregnancy than to Addisonian pernicious anemia.

University of Utah College of Medicine
Salt Lake City, Utah

315. CARTWRIGHT G. E., TATTING B., ROBINS J., FELLOWS, N. M., GUNN F. D., and WINTROBE, M. M.: *Hematologic manifestation vitamin B₁₂ deficiency in swine*, Blood 6: 867 Oct. 1951

Of 39 pigs not receiving B₁₂, 13 failed to develop anemia, 16 developed a mild anemia and 10 developed a moderately severe anemia. The anemia when present normocytic and in 24 pigs was accompanied by a moderately severe neutropenia. There was increase in no blasts in sternal marrow. Hematologic alterations neither consistently nor completely corrected by the administration of vitamin B₁₂ in spite of the fact that nitro and marked reticulocyte increases followed.

These results are in contrast to those obtained with an experimentally produced deficiency of B₁₂ in man. Such animals develop macrocytic anemia, leukopenia, a macrocytoblastic type of bone marrow

Megaloblastic anemia may not have developed in B₁₂-deficient pigs because (1) the deficiency was sufficiently severe to result in such a change, or because PCA prevents the development of megaloblastic anemia even in the absence of vitamin B₁₂.

316. CARTWRIGHT G. E., and WINTROBE, M. M.: *Experimental production of nutritional macrocytic anemia in swine*, Am. J. Physiol. 187: 773-774 1951 (In Soc. Proc.)

"A severe macrocytic anemia, leukopenia and thrombocytopenia have been produced in 56 swine deficient in pteroylglutamic acid. The bone marrow of such animals was hyperplastic and there was an increase in large immature nucleated red cells (macronuclear blasts) of a type which resembled but which were identical with the megaloblasts of pernicious anemia. Hematologic manifestations of this deficiency appear in the presence of extrinsic factor in the diet and not prevented or alleviated by the administration of feed liver extract or crystalline vitamin B₁₂. In an attempt to produce a deficiency of vitamin B₁₂ a total of 70 were fed a purified diet containing soybean alpha protein in place of casein. Growth of the animals on the diet without added vitamin B₁₂ was retarded as compared with the growth of animals on the same diet with this vitamin. Anemia was not a prominent feature of the deficiency syndrome and when present was

mild in degree and normocytic in type. Abnormalities in the nucleated red cells in the sternal marrow were not present. A combined deficiency of both pteroylglutamic acid and vitamin B₁₂ has been produced in 21 pigs. These animals developed a severe macrocytic anemia, leukopenia and slight thrombocytopenia. Pernicious anemia-like megaloblasts were observed in small numbers in the bone marrow. The majority of the nucleated red cells were macrocytomegaloblasts. The anemia responded partially to crystalline vitamin B₁₂ and completely to pteroylglutamic acid. The role of pteroylglutamic acid and vitamin B₁₂ in normal erythropoiesis is discussed.

University of Utah College of Medicine
Salt Lake City, Utah

- 317 CARTWRIGHT G. E., TATTING R., KURTH, D., and WINTROBE, M. M.: *Experimental production of nutritional macrocytic anemia in swine V Hematologic manifestations of a combined deficiency of vitamin B₁₂ and pteroylglutamic acid*, Blood 7 922-1004, Oct. 1952.

Authors' summary "A total of 20 swine were fed a diet adequate in all known respects except that soyabean protein was substituted for casein, succinylmaltolactose and a folate acid antagonist were added, and vitamin B₁₂ and pteroylglutamic acid were withheld from the vitamin supplement.

"The animals developed macrocytic anemia, leukopenia and neutropenia, accompanied by erythroid hyperplasia of the bone marrow. The erythroblasts consisted mainly of immature macrocytomegaloblasts but a few atypical megaloblasts were also observed.

"The anemia responded rapidly and completely to the administration of both vitamin B₁₂ and pteroylglutamic acid. The administration of pteroylglutamic acid alone resulted in an immediate return of the blood and bone marrow to within normal limits but after several months there was a partial hematologic relapse in spite of continued therapy with this vitamin. The administration of vitamin B₁₂ alone resulted in only partial remission of the anemia and the bone marrow remained macrocytomegaloblastic although the megaloblasts tended to disappear.

"Growth of the animals was stimulated by the administration of either vitamin but the administration of both vitamins simultaneously resulted in the greatest rate of growth.

"No manifestations of neurologic disturbances or of increased pigment excretion were observed in the deficient swine."

University of Utah College of Medicine
Salt Lake City, Utah

Dogs

318. RUEGAMER, W. R., BRICKSON, W. L., TORBET N. J., and ELVEHJEM, C. A.: *Response of dogs to liver extracts containing the pernicious anemia factor J* Nutrition 36 425-435, Oct. 1948.

Liver extracts rich in pernicious anemia factor were effective in restoring the blood picture and general health of young dogs fed a p-aminocaproic acid, containing 1% sulfanilamide. Combination of liver extracts with folate acid produced complete recovery

Monkeys

- 319 MAY, C. D., SUNDBERG R. D., SCHAAER, F., LOWE, C. U., and SALMOV R. J.: *Experimental nutritional megaloblastic anemia: relation of ascorbic acid and pteroylglutamic acid J Nutritional Dia. Child. 82 282-309 Sept. 1951*

Authors' summary "Megaloblastic anemia has been produced regularly in monkeys by feeding milk diets deficient in ascorbic acid. Monkeys fed the same milk diets supplemented with adequate ascorbic acid remain in good health for prolonged periods and maintain normal blood and bone marrow. This experimental megaloblastosis is virtually indistinguishable from that seen in megaloblastic anemia in humans, particularly the form encountered most frequently in infancy. This experimental megaloblastosis can be eliminated or prevented by pteroylglutamic acid or folic acid without the aid of ascorbic acid. It can also be cured by ascorbic acid alone. Vitamin B₁₂ will neither cure nor prevent it. This experimental megaloblastosis is due to a disturbance in the metabolism of pteroylglutamic acid caused by a deficiency of ascorbic acid. Application of this knowledge to elucidation of pathogenesis of megaloblastic anemia in infancy is mentioned."

University of Minnesota Medical School
Minneapolis, Minn.

320. SMITH, S. C., and ELVEHJEM, C. A.: *An inter relation of pteroylglutamic acid, vitamin B₁₂ and the monkey anti-anemia factor J Nutrition 45 47-59 Sept. 1951.*

Authors' summary "A deficiency of PCA was created in young rhesus monkeys by means of a purified diet. Signs characteristic of a lack of the monkey anti-anemia factor accompanied those of the primary deficiency. Hemolysis of the monkey anti-anemia factor deficiency syndrome was obtained by the oral administration of PCA at a level 10 times the minimum daily requirement.

"Remission for a limited period was also obtained with a 60% methanol extract of liver supplying only 4 µg. each of PCA and vitamin B₁₂ per day. Eventually PCA had to be supplied to maintain normal growth rate and blood picture, but it was required in much smaller amounts than when the methanol extract of liver which is a good source of the monkey anti-anemia factor was not employed.

"Vitamin B₁₂ produced responses only in the least deficient monkeys and probably acted in conjunction with vitamin C to stimulate the synthesis of PCA.

"A relationship exists among PCA, vitamin C, vitamin B₁₂ and the monkey anti-anemia factor although the latter two substances are not ordinarily required in the diet of the normal monkey.

"At high levels PCA appears to be capable of satisfying any requirement that may exist in a PCA-deficient monkey for vitamin B₁₂ or the factor or factors present in methanol extract of liver (the monkey anti-anemia factor) "

University of Minnesota
Minneapolis, Minn.

321. POPPEN A. J., GREENBERG L. D., and RINEHART J. F. *The blood picture of pyridoxine deficiency in the monkey* Blood 7: 436-444, April 1952.

Authors summary and conclusions "Pyridoxine deficiency in the monkey results in a severe anemia characterized by hypochromia, increase in mean diameter decrease in mean cell thickness, dehydration, the appearance of target cells and increased resistance to hemolysis.

"The administration of pyridoxine results in restoration of hematologic values to normal, correction of dehydration and disappearance of target cells.

"Further evidence is offered relating target cells to dehydration and, in addition, possibly to hepatic damage.

"A delayed macrocytic anemia which was not corrected by low doses of vitamin B₁₂, folic acid or iron is described in control animals. This anemia occurred chronologically one to two years later than the induced anemia of pyridoxine deficiency and is not necessarily related. The cause of this anemia is unknown.

"Normal hematologic data on twenty-one *M. rhesus* monkeys is presented."

University of California School of Medicine
San Francisco, Calif.

322. MAY C. D., HAMILTON, A., and STEWART, C. T. *Experimental megaloblastic anemia and scurvy in the monkey. IV. Vitamin B₁₂ and folic acid compounds in the diet, liver, urine and feces and effects of therapy* Blood 7: 978-991, Oct. 1952.

Authors conclusions: "The concentration of vitamin B₁₂ and folic acid compounds in the diet, liver, urine and feces of control and scorbutic monkeys was determined and compared with the type of hematopoiesis in the bone marrow.

"Monkeys fed milk diets devoid of ascorbic acid regularly developed a marked deficiency of folic acid compounds and megaloblastic anemia as a complication of scurvy.

"Megaloblastosis in the marrow did not develop in the scorbutic monkey unless the level of free folic acid in the liver was low.

"Low levels of free folic acid in the liver were found only with low levels of total folic acid, but the level of free folic acid was sometimes found to be normal although the level of total folic acid was low.

"The focal content of vitamin B₁₂ and folic acid compounds was the same in control and scorbutic monkeys.

"Urinary excretion of folic acid did not prove to be a practical index of tissue stores.

"The effects of various procedures and therapeutic substances on the bone marrow and the liver content of vitamin B₁₂ and the folic acid compounds was studied.

"Treatment of the scorbutic megaloblastic monkey with either folic acid or L-ascorbic acid restored the marrow to normal and caused accumulation of folic acid and folic acid in the liver.

"The effect of L-ascorbic acid was quite specific as D-ascorbic acid and D-glucoscorbic acid had no effect on the marrow or the liver concentration of folic acid compounds."

University of Minnesota College of Medical Sciences
Minneapolis, Minn.
State University of Iowa
Iowa City, Iowa

323. SUNDBERG R. D., SCHAAR, F., and MAY C. D. *Experimental nutritional megaloblastic anemia. II Hematology* Blood 7: 1143-1181, Dec. 1952.

Authors conclusions "Megaloblastic anemia has been produced in monkeys by feeding milk diets, low in folic acid, and deficient in ascorbic acid.

"Vitamin B₁₂ was not an effective therapeutic or prophylactic agent.

"Folic acid caused prompt reversion of the megaloblastic marrow to a normoblastic marrow. If ascorbic acid was also given, the monkey survived and the hemoglobin and erythrocyte levels returned to normal.

"Ascorbic acid with no source of folic acid other than the experimental diet caused reversion of the marrow from megaloblastic to normoblastic. The differential counts suggest that this process was slightly slower than the similar change which occurred with folic acid.

"Folic acid proved an effective therapeutic agent in doses smaller than the doses of folic acid which proved effective in these experiments.

"Semi-starvation did not produce megaloblastic anemia.

"The transformation of the megaloblastic to the normoblastic marrow has been discussed.

"The type, sequence and rate of changes in the marrow pattern in response to various therapeutic agents has been illustrated, presented in graphic form and discussed."

University of Minnesota College of Medical Sciences
Minneapolis, Minn.

LEUKEMIAS

324. HEILMEYER, L. *"Perniciöse Anämie" als Initialphase einer akuten erythroleukämie (Pernicious anemia as the initial phase of acute erythroleukemia)* Schweiz. med. Wochenschr. 80: 1122-1124, Oct. 14, 1950.

Two patients with initial diagnoses of pernicious anemia are described. Liver extract therapy in one and

Hepar glandol (each ampul of which contains 15 mcg. of vitamin B₁₂ and 10 mg. of folic acid) in the other were ineffective. Subsequent sternal puncture studies revealed that true myeloblastic leukemia had developed. The diagnostic difficulties in this type of patient are discussed. The author states that the initial megaloblastic anemia in these patients was not of the pernicious type, but

rather to be part of a malignant transformation of the blood picture, hence the lack of response to vitamin B₁₂.

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Freiburg, Germany*

325. BARNARD R. D., and FOX, H. L.: *Innocuity of protracted oral administration of special fermentation concentrates of B₁₂ as demonstrated in a patient with acute leukemia*, Ohio State M. J. 46: 784-786, Aug. 1950.

The authors describe a patient with acute leukemia who was released from the hospital on Dec. 18, 1949 in anticipation of rapid deterioration and death. On the day of discharge, the following dietary supplement was instituted, to be taken four times a day: 20 Gm. of crude linseed oil emulsified in milk with Tween 80, and 10 Gm. of crude fermentation concentrates from an antibiotic-producing *Streptomyces* strain. The patient began to improve on this regimen and, as of April 1950, he apparently was normal both clinically and hematologically. During this period his daily dietary supplement contained 240 to 320 mcg. of vitamin B₁₂ and 9 to 12 mg. of antibiotic (in terms of streptomycin activity). In April the linseed oil-Tween 80 emulsion was discontinued, and a concentrate from a non-antibiotic-producing *Streptomyces* strain was given in doses corresponding to 550 mcg. of vitamin B₁₂ a day. Clinical, but not hematologic, deterioration followed. Thereafter the daily diet was supplemented by a concentrate supplying 80 mcg. of vitamin B₁₂ and 3 mg. of antibiotic, and by 750 mg. of terramycin (from *Streptomyces rimosus*). As of July 1950, the patient was receiving 100 mg. of terramycin daily. He was now in the eighth month of remission.

Altogether the senior author has recorded 29 cases of acute leukemia in which dietary supplements from antibiotic-producing *Streptomyces* species have produced 11 remissions. The authors believe the remissions to be due to the coliform-suppressing antibiotic present in the fermentation concentrate. With regard to vitamin B₁₂, the remission in the case described was believed to be probably coincidental to its administration. The results, however, imply the complete innocuity of massive oral doses of vitamin B₁₂.

The authors consider that, in the acute leukoblastic process, the blast is a result rather than a cause of the disease. They do not use myelosuppressive therapy. They state that the tonic and supportive properties of factors associated with vitamin B₁₂ may eventually play an important part in the management of malignant leukoblastoses.

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326. BARNARD R. D. *Streptomyces fermentation derivatives in acute leukemia*, *Lancet* 1: 1157-1159 May 26, 1951.

The author reports his experiences in treating acute leukemia with a fermentation by-product of *Streptomyces griseus*, and describes 3 illustrative cases. The first patient, a 17 year old girl, was treated with penicillin for a "viral" pneumonia. When she did not respond, and the spleen became palpable, blood and marrow samples were taken which indicated acute myeloblastic leukemia. Strep-

tomycins residue (with a vitamin B₁₂ equivalence of 6 mcg. per gram, and 220 "units" of streptomycin, equivalence in terms of *Bacterium coli* suppression) was given, 40 Gm. divided into four daily doses. Parenteral penicillin was discontinued after 24 hours, with the patient much improved, with increased appetite and appearance of health. Temperature became normal, and the patient got out of bed within 72 hours after the treatment was begun. The patient refused the residue mixture about two weeks after treatment was begun, and a relapse occurred 48 hours after the treatment was stopped. Death, apparently due to a cerebral hemorrhage, occurred 19 days after therapy was discontinued.

The patient had been ingesting, in the residue mixture, about 0.5 mg. of B₁₂ and 11 mg. of antibiotic daily. "Blast" forms had declined and platelets had increased markedly during residue therapy though the erythrocyte level remained the same.

The second patient, a 12 year old boy was also treated with penicillin for a "viral" pneumonia. When acute leukemia was diagnosed, 10 Gm. daily of *Streptomyces* residue containing 55 mcg. of B₁₂ per Gm., but without the coliform suppressing factor was given orally. The patient declined during the next 12 days, and 15 days after the residue treatment was begun, 1.5 Gm. daily of terramycin hydrochloride was substituted for the antibiotic-free residue. Two days later the temperature returned to normal, and six days afterwards the child went home in clinical remission. The child died later possibly as the result of a transfusion.

It is possible that, since the child received over 0.5 mg. of vitamin B₁₂ daily the vitamin was stored, and its combined activity was responsible for the remission. The author feels, however, that the action of the antibiotic element against the increased number of proteolytic organisms in the intestinal tract was responsible for the remission.

The third patient, a 69 year old man, was hospitalized with acute prostatic, and later an acute lymphoblastic leukemia was diagnosed. Aureomycin administration, 1 Gm. daily was begun on admission. Transurethral resection was performed the second week, and when 0.25 mg. of terramycin and 0.25 mg. of chloramphenicol daily were substituted for the aureomycin, bleeding stopped and fever abated. Stools became odorless, with a predominantly gram-positive intestinal flora. Eight months after the apparent remission, the patient was in the best of health. On the combined terramycin and chloramphenicol treatment, and later on 100 mg. of terramycin daily the erythrocyte count climbed steadily toward normal. Numbers of lymphoblasts declined rapidly on the same treatment. In this case terramycin apparently eliminated an aureomycin-resistant strain of *Bacterium aerogenes*.

The three cases reported are illustrative of the 50 patients the author has treated. Convincing statistics on all the patients are lacking, since other factors, such as transfusions and anti-folate acid treatment, confuse the picture. There were only six one-year survivals among 50 patients.

In an addendum, the author mentions that Kersey (*Proc. Soc. Am. Bact.*, March 20, 1951) reports survival

of intestinal lactobacillus with small doses of terramycin, but not with large doses.

Author's summary "Food supplements obtained by fermentation with *Streptomyces griseus* have been given by mouth to patients with acute leukemia. In some cases the clinical condition has greatly improved, but the blood picture has not shown corresponding changes.

"Though the products used are rich in vitamin B₁₂ their effect in leukemia is more probably related to their content of antibiotic. *Streptomyces*-derived antibiotics alter the intestinal flora, suppressing the collagenogenic organisms and the clinical remissions seem to last until antibiotic resistant strains emerge in repopulate the gut. This event was apparently postponed in patients whose large intake of milk promoted the growth of lactobacilli.

"Untoward effects on salt and water balance have been observed in some cases treated with streptomycetes fermentation products, and more experience will be needed before any satisfactory régime for their use can be evolved."

Lancet
Lanc. Edinb., N. Y.

- 327 BARNARD R. D., KOPET S. J., and STAHL, A. E. Erythremia ("polycythemia") during massive, oral, *Streptomyces*-derived B₁₂ therapy. *Ann. Allergy* 9 360-367 May-June 1951.

Two cases are reported in which the patients received an "oral grade B₁₂" derived from the residue of an antibiotic-nonproducing *Streptomyces* strain. Both patients developed transient erythremia, but also experienced a marked abatement of allergic symptoms and a stabilization of a malignant leukoblastic process. In one case the primary diagnosis was Hodgkin's lymphoma and in the other agnogenic myeloid metaplasia.

Quanta, N. Y.

328. WILSON S. J.: Observations on the effect of various folic acid antagonists on acute leukemia. *Blood* 6 1002-1012, Nov. 1951.

Prolonged but temporary remissions have occurred in patients with acute leukemia treated with folic acid antagonists. In the present study 70 patients were treated, 65 of whom survived for a sufficient length of time to evaluate the effect of the drugs. Folic acid antagonists used were aminopterin, 8-methopterin, amino-an-fol, and 8-ninopterin. Leukemias studied were acute lymphatic, monocytic and acute myelogenous leukemia. Eleven pa-

tients showed good response with remissions of 29 to 490 days. Of these 11 10 had lymphatic leukemia (8 of them were children under 10 years of age). Management of toxic reactions included vitamin B₁₂ and liver extracts in most cases. Discontinuance of the antagonist at first signs of toxicity seemed the most effective procedure. Folic acid was not used.

The summary reads, in part "When a response occurred, a rather definite hematologic pattern was noted. An increased platelet count in most instances was the first evidence of regeneration and occurred in about the third or fourth week. The neutrophilic polymorphonuclear leukocytes began to regenerate at about the same time and an increase in their number was followed by a rise in the erythrocyte count. Observations of the bone marrow indicated that although excellent clinical and hematologic remissions might occur primitive leukemic cells were still present. In some instances megakaryoblasts were observed in addition to a peripheral macrocytosis and anisocytosis of erythrocytes. Toxic manifestations were common. These included glossitis, ulceration of the oral cavity, nausea, vomiting, diarrhea and alopecia. In one instance there was ulceration of the entire gastro-intestinal tract, including the esophagus and colon. Hematologic toxic reactions included thrombocytopenia, leukopenia and anemia. Aplasia of marrow tissue was observed in 1 instance. In many instances the margin of safety between a toxic reaction and death was indeed small."

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Kansas City, Kan.

- 329 WELSH, L. Failure of massive doses of vitamin B₁₂ in acute leukemia. *Brit. M. J.* 2 1133, Nov. 22, 1952.

Twenty-five patients with acute leukemia (10 myeloblastic, 6 undifferentiated cell type, 5 lymphoblastic, 4 monocytic) were treated with daily 1-mg. doses of vitamin B₁₂. Therapy started at diagnosis and (in all but seven cases) continued until death. Transfusions and antibiotics were given as needed, but other remedies such as X-rays, cortisone or corticotropin were not given. None lived more than five months after diagnosis and beginning of treatment. There were no complete remissions. Six patients experienced partial remissions and 10 others reported feeling better. The author states: "There is no evidence from this series to suggest that the prognosis or clinical course of acute leukemia is altered by adding vitamin B₁₂ to other treatment."

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Oxford, England

ANIMAL STUDIES

330. SKIPPER, H. E., BELL, M., and CHAPMAN J. B. Partial reversal of the antileukemic action of folic acid antagonists by nucleic acids. *Cancer* 4 357-359 March 1951.

In a series of experiments in leukemia mice it was shown that desoxyribonucleic acid and ribonucleic acid will partially reverse the antileukemic action of folic acid antagonists. In a separate experiment it was noted that vitamin B₁₂, added to desoxyribonucleic acid, pro-

vided an even greater reversal of an antagonist (8-methopterin) than did DNA alone.

Southern Research Institute
Birmingham, Ala.

- 331 SKIPPER, H. E., CHAPMAN J. B., and BELL, M. Partial reversal of the anti-leukemic action of folic acid antagonists by vitamin B₁₂. *Cancer Research* 11 161-163, March 1951.

Eight separate experiments with controls were run on 228 mice injected with Ak 4 leukemia. Ninety mice were treated with the folic acid antagonists aminopterin or A-methopterin, and 188 were treated with the antagonists plus either B_{12} concentrate or crystalline B_{12} . Results showed that the addition of B_{12} partially reversed the anti-leukemic action of folic acid antagonists. With A-methopterin, B_{12} shortened the life span an average of 2.6 days.

"It would appear that B_{12} in some obscure fashion is helping to provide chemical moieties (nucleic acid precursors) which are deficient in the leukemic cells of mice treated with folic acid antagonists. Such an interpretation is compatible with Shive's observation that B_{12} is involved in the biosynthesis of purines (or derivatives) as well as thymine in certain bacteria."

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Birmingham, Ala.*

332. SKIPPER, H. E., CHAPMAN, J. B., BOYD G. A., RISER, W. H., JR., and BELL, M. *Preferential incorporation of formate carbon into leukemic*

blood cells as indicated by autoradiography Proc. Soc. Exper. Biol. & Med. 77: 849-854, Aug. 1951.

One hour after injection of C^{14} -formate into mice with advanced leukemia, 90 per cent of the leukemic cells, in comparison to about 5 per cent of the more active lymphoid elements, are highly active. In the discussion, consideration is given to the possible part played by citrovorum factor coenzyme (thought to be involved in formate transfer) in normal and leukemic cells, by folic acid antagonists which inhibit the conversion of folic acid to citrovorum factor and inhibit incorporation of formate into nucleic acids, by folic acid deficiencies which inhibit incorporation of formate into proteins, by the anti-leukemic action of folic acid inhibitors, by the reversal of the antileukemic action of folic acid antagonists, by folic acid or citrovorum factor and by the partial reversal of this action by deoxyribonucleic acid or vitamin B_{12} , and by other factors.

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Nervous System

NEUROLOGIC COMPLICATIONS

SUBACUTE COMBINED DEGENERATION

- 333 UNGLEY C. C. *Anti-anæmic substances from liver* Lancet 1 771-772, May 15 1948 (in Letters to the Editor)

In the past six months, 3 pernicious anemia patients with subacute combined degeneration of the cord have been treated for periods of 21, 12, and 10 weeks with the highly purified material prepared from liver by the Glaxo Laboratories. In each case neurologic symptoms and signs have decreased progressively. The degree of improvement has been in no way inferior to that observed in comparable cases in which crude liver extract has been administered.

- 334 BERK, L., DENNY-BROWN D., FINLAND M., and CASTLE, W. B. *Effectiveness of vitamin B₁₂ in combined system disease: rapid regression of neurologic manifestations and absence of allergic reactions in a patient sensitive to injectable liver extracts* New England J. Med. 239 328-330, Aug. 26, 1948.

A patient with pernicious anemia and combined neurologic symptoms was treated with crystalline vitamin B₁₂. Hematologic remission and rapid improvement in the neurologic symptoms occurred after B₁₂ therapy. This patient was sensitive to liver extracts and had received folic acid therapy irregularly on previous occasions. The vitamin B₁₂ was given intradermally (initial dose) and intramuscularly in a daily dose of 5 mcg. This amount is forty five times as great as that contained in 0.01 cc. of liver extract. On the ninth day of treatment the drug had to be stopped because of lack of supply. The patient's reticulocyte and red cell count had increased and there was excellent improvement of the neurologic disorder. A setback in neurologic improvement occurred when the drug was discontinued. After seven days, it was again administered, this time in doses of 5 mcg. Intramuscularly three times a week, and steady improvement has resulted. Other than a small transient wheal at the site of intradermal injection, there were no allergic reactions attributable to vitamin B₁₂, which indicates that the B₁₂ content is not responsible for sensitivity reactions caused by liver extracts.

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Barnes City Hospital
Barnes, Minn.

- 335 SPIES, T. D., STONE, R. E., GARCIA LOPEZ, C., MILANES, F., ARAMBURU T., and LOPEZ TOCA, R. *The association between gastric achlorhydria and subacute combined degeneration of the spinal cord*, Postgrad. Med. 4 89-95, Aug. 1948.

All of 160 adult patients suffering from macrocytic anemia (pernicious anemia, nutritional macrocytic anemia, macrocytic anemia of pregnancy, tropical sprue) who were included in a study of the therapeutic effects of folic acid showed a hematologic response to this vitamin associated with remarkable clinical improvement. However in 28 of the 33 cases of pernicious anemia

studied, subacute combined degeneration of the spinal cord developed, and progressed despite the folic acid, until liver extract was administered. Massive liver extract therapy gave prompt relief. Each of these 28 patients had gastric achlorhydria prior to folic acid treatment. None of the patients with other types of macrocytic anemia had achlorhydria either before or during folic acid therapy and none developed subacute combined degeneration of the cord, which suggests a significant relationship between these two states.

Three patients suffering from Addisonian pernicious anemia with achlorhydria and subacute combined degeneration, not included in the above series, were given 15 mcg. of vitamin B₁₂ intramuscularly. All showed prompt and remarkable subjective improvement. In the most acute case there was considerable improvement also in objective physical findings within two weeks, and in another case slight objective improvement was observed in a similar period.

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Buenos Aires, Cuba

- 336 SPIES, T. D., STONE, R. E., KARTUS, S., and ARAMBURU T. *The treatment of subacute combined degeneration of the spinal cord with vitamin B₁₂*, South. M. J. 41 1030-1031 Nov. 1948.

In 3 patients with macrocytic hyperchromic anemia and subacute combined degeneration of the spinal cord, the neurologic status improved following the injection of vitamin B₁₂. One case is reported in detail—that of a 48 year old man whose neurologic condition was considerably improved after he had received four injections of 25 mcg. of vitamin B₁₂ given at intervals of 48 hours. His blood response also was good.

Northwestern University
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St. Mary's Hospital
Birmingham, Ala.

- 337 STONE, R. E., and SPIES, T. D. *Vitamin B₁₂ and subacute combined degeneration of the spinal cord* Rev. internat. de vitaminol. 20 228-233, 1948.

Three patients with pernicious anemia and subacute combined degeneration of the spinal cord who had been examined frequently over a long period were treated with vitamin B₁₂. In each instance the symptoms improved, and in 2 some of the abnormal physical findings were reversed. One of these cases is reported in detail. A 31 year old Negro woman was admitted to the Hillman Hospital in relapse a week after folic acid treatment had been discontinued. An intramuscular injection of 15 mcg. of vitamin B₁₂ was given. Her reticulocyte count rose from 2 to 13 per cent in seven days, fell to 2.2 on the thirteenth day and was 3.2 per cent on the sixteenth day when she was discharged. The initial blood counts of 2.66 million red cells and 3,400 white cells, and hemoglobin 65 per cent, increased to 3.39 million, 3,900, and 77 per cent, respectively. Soreness of the tongue responded quickly, neurologic symptoms improved, and strength in-

ceased. When dismissed from the hospital the patient still complained of numbness of her feet and finger tips and slight stiffness of one knee. Five days later there had been no further improvement, and a second dose of vitamin B₁₂ (9 mcg.) was given. Fourteen days later the blood values were 3.62 million red cells, 68 per cent hemoglobin, and 2.9 per cent reticulocytes, which were greater than had been obtained previously with folic acid or liver extract. Residual paresthesias persisted, but the patient gained weight and was able to be increasingly active.

338. HALL, B. E., and CAMPBELL, D. C.: *Vitamin B₁₂ therapy in pernicious anemia. II Effect on the general clinical and neurologic manifestations—preliminary report*, Proc. Staff Meet., Mayo Clin. 23: 591-595, Dec. 8, 1948.

Clinical and neurologic responses to vitamin B₁₂ in the same 11 patients with pernicious anemia in relapse whose hemopoietic responses were reported in Part I of this paper (Abstract 87) are presented here. The patients were observed for one to three and a half months. Improvement in strength, mental alertness and appetite, gain in weight, and disappearance of glossitis were noted. A patient with peripheral neuritis but without involvement of the spinal cord reported disappearance of paresthesias in one and a half months; 2 others noted improvement in one month, and further improvement may be expected. Five of 6 patients with peripheral neuritis and combined degeneration of the cord showed neurologic improvement, which was unusually rapid in 3.

339. JONES E.: *Vitamin B₁₂*, Am. Practitioner 3: 891-892, March 1949

The author briefly reviews the literature on vitamin B₁₂ and states that his personal observations on 2 patients agree with the reports that vitamin B₁₂ is of value in the treatment of combined system disease.

340. PATTERSON J. F., STAUFFER, R., and FROMMIN, A. M.: *The effect of vitamin B₁₂ on hematologic and neurologic disorders in pernicious anemia—report of a case*, Bull. New England M. Center 11: 120-126, June 1949

341. METTIER, S. R., MCBRIDE, A., and TAT R.: *The effect of vitamin B₁₂ on the anemia and combined system disease of addisonian pernicious anemia*, California Med. 71: 21-27 July 1949

Histories of 8 cases of pernicious anemia are presented. The 4 uncomplicated cases responded equally well to 50 or 25 mcg. of vitamin B₁₂ given intramuscularly. When the reticulocyte count had fallen, a secondary response was induced in only one of these patients by an injection of 25 mcg. indicating that the initial treatment had been suboptimum in this patient but optimum in the others. Improvement was maintained in the patients with injections of 25 mcg. of vitamin B₁₂ every two weeks. The erythrocytes had returned to normal range within the 60 days in the 3 patients who continued treatment. The fourth discontinued treatment and had a return of the anemia, which responded to renewed injections of B₁₂.

Two of the 8 patients who had pernicious anemia complicated by combined system disease showed an optimum, and the third a suboptimum, hemopoietic response to injections of vitamin B₁₂. Minor paresthesias and loss of vibratory sense disappeared in one of these patients and disturbances of a more serious degree in the others improved greatly.

An eighth patient had been satisfactorily treated with liver extract until she became pregnant. She then became sensitive to liver extract, treatment was discontinued, and anemia recurred. Following the injection of 25 mcg. of vitamin B₁₂ there was a satisfactory response of the blood, and there were no signs of drug sensitivity.

The hemopoietic response in three patients was accompanied by change of the bone marrow from megaloblastic hyperplasia to normoblastic distribution. Maintenance injections of vitamin B₁₂ may be from 30 to 50 mcg. at intervals of one month, depending on the individual case.

342. KRUSEN F. H., HALL, B. E., and WOLTMAN H. W.: *Coordination exercises and vitamin B₁₂ for combined degeneration of the spinal cord in pernicious anemia*, A.M.A. Scientific Assembly Official Program, June 1949 p. 195-196.

"If degeneration of the lateral and posterior columns of the spinal cord associated with pernicious anemia is diagnosed early and treated promptly decided improvement will often occur. Treatment consisting of correct administration of vitamin B₁₂ combined with proper instruction in coordination exercises has proved extremely effective. The results obtained in such management of 10 cases of combined degeneration of the spinal cord have been uniformly encouraging.

Endocrine Note.

343. HALL, B. E., KRUSEN F. H., and WOLTMAN H. W.: *Vitamin B₁₂ and coordination exercises for combined degeneration of the spinal cord in pernicious anemia*, J.A.M.A. 140: 257-260, Sept. 24, 1949

The importance of coordination exercises in conjunction with vitamin B₁₂ administration in patients with spinal cord degeneration associated with pernicious anemia should not be overlooked. Treatment with vitamin B₁₂ and exercise must be started early before the axons within the brain and cord have been destroyed, in order to obtain restoration of function.

The vitamin should be given in doses of 10 mcg. intramuscularly each day or every second day for three to six months. Maintenance doses of 10 to 20 mcg. per week should follow the more intensive treatment. These doses are in excess of those needed by patients with pernicious anemia without involvement of the nervous system, and it is possible that even larger doses would be more effective. The exercises should be done during the period of vitamin B₁₂ therapy.

Twelve patients having subacute combined degeneration of the spinal cord associated with pernicious anemia were given combined parenteral administration of vitamin B₁₂ and coordination exercises. In three to nine months,

neurologic manifestations had disappeared or abated, in the majority of cases. Results are summarized in the accompanying table.

Neurologic symptom	No. cases	Recovered	Improved	Not improved
<i>Parosmia</i>				
Upper limbs	9	3	4	6
Lower limbs	11	3	5	8
<i>Ataxia</i>				
Trunk	10	6	4	6
<i>Vibration sense</i>				
Upper limbs	12	3	7	4
Lower limbs	10	4	3	1
<i>Position sense, toes</i>				
Right	11	0	3	8
<i>Quadriceps muscle</i>				
Right	12	2	4	6
<i>Thighs nerve pain</i>				
Right	11	3	3	7
<i>Shin pain</i>				
Right	9	4	2	9
<i>Clonus</i>				
Right	1	1	0	0

- 344 MURRAY M. Y., and PROPP S.: *Vitamin B₁₂ effective in pernicious anemia with combined system disease and liver sensitivity*. New York State J. Med. 49: 2570-2572, Nov. 1, 1949

Sensitivity to injected liver extract is not uncommon. It was found in 17 per cent of 996 patients with pernicious anemia treated by Schwartz and Legere (*Blood* 1: 307, 1946). In the case presented here, a patient with pernicious anemia developed severe sensitivity to liver extract, which was unrelieved by desensitization, antihistaminic drugs, and various other measures. Folic acid was substituted for the liver extract and although well tolerated failed to prevent glossitis and the progress of serious gastrointestinal symptoms. After about a year and a half of folic acid therapy signs of combined system disease appeared and advanced rapidly. Folic acid was withdrawn, and 5 mcg. of vitamin B₁₂ was given intradermally. As no untoward reaction occurred, the patient was thereafter given 5 mcg. of this vitamin intramuscularly every day for six days, then 10 mcg. daily for nine days, followed by 5 mcg. three times a week, and finally 10 mcg. once a week. Under this treatment a remarkable improvement took place in the combined system symptoms, the gastrointestinal symptoms disappeared, and the blood values returned to normal.

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- 345 UNGLEY C. C.: *Subacute combined degeneration of cord. I. Response to liver extracts. II. Trials with vitamin B₁₂. Appendix: quantitative method of assessing neurological status*. Brain 72: 382. Sept. 1949 (abstr. J.A.M.A. 143: 899, May 27, 1950)

The author has attempted to determine the nature of the agent in liver which is effective in subacute combined degeneration of the cord. Successful trials were made with a red crystalline anti-pernicious anemia factor, vitamin B₁₂, one of the red pigments contained in Lester Smith's less pure earlier material. Eight patients with subacute combined degeneration were studied. The first 4 patients, who were given the less pure material initially, have been treated for 14 to 19 months, for the past 13 of which they have received the pure substance. The remaining 4 patients treated for 9 to 13 months, were given only the crystalline material. The improvement after vitamin B₁₂ therapy was compared with the "expected" improve-

ment based on results obtained in 44 patients treated with liver extract. Four of the 8 patients exceeded the expected rate of improvement, 2 attained it and 2 fell just short of it. Vitamin B₁₂ is therefore as effective as liver extract not only in pernicious anemia but also in subacute combined degeneration. Except in patients who are sensitive to liver extract, the author sees no advantage in using pure vitamin B₁₂. Weekly doses of 40 mcg. of vitamin B₁₂ or the equivalent amount in the form of liver extract is recommended for the first six months, followed by 20 mcg. weekly as a maintenance dose thereafter.

346. UNGLEY C. C.: *Liver extracts*, Lancet 1: 708, 1949

No significant difference was found between crude and "refined" liver extracts in treatment of 44 cases of subacute combined degeneration. Furthermore, vitamin B₁₂ was as effective as liver extract, crude or refined, in this disease.

- 347 QUESTIONS AND COMMENTS: *Subacute combined degeneration*, Brit. M. J. 2: 1539, Dec. 31, 1949

Treatment with liver extract or with vitamin B₁₂ is recommended for a case of subacute combined degeneration of the cord with predominantly neurologic symptoms. With regard to vitamin B₁₂, it is stated that recent investigations have suggested its value in this condition but that as yet dosage and long-term effects have not been fully reported. It is suggested that doses of 40 to 60 mcg. intramuscularly once or twice weekly be tried. A warning is given not to administer folic acid under any circumstances either alone or in combination with any other preparation. Administration of dilute hydrochloric acid and glycerin, peptin, supplementary vitamins, especially B complex, and also ascorbic acid, a full normal diet, and massage and exercise are suggested as ancillary treatment. It is further stated that the response will vary according to the severity of the initial condition, the intensity and persistence of treatment, and the individual sensitivity of the patient, but that improvement should be noticeable in the first few weeks, particularly as the blood count returns to normal, after which it should be progressive although probably slow. Paresthesias and some spasticity may persist for a long time.

348. JEQUIER, M., and HEMMELER, G.: *Treatment of pernicious anemia and fundular myelosis: danger of folic acid*, Praxis 39: 63. Jan. 26, 1950 (abstr. J.A.M.A. 143: 1215, July 29, 1950)

Five patients with pernicious anemia, who had responded favorably to liver therapy were given folic acid. All of the patients developed a neuroanemic syndrome after several months of treatment with folic acid. Folic acid was withdrawn and treatment was continued with liver extract and vitamin B₁₂. The folic acid did not exert any effect on glossitis which some of the patients presented. The authors' observations warrant the conclusion that folic acid does not prevent the occurrence of fundular myelosis or its aggravation. Folic acid seems rather to induce involvement of the nervous system.

- 349 BERGER, H.: *Vitamin B₁₂ in Addisonian pernicious anemia in a liver sensitive person*, New York State J. Med. 50: 331-332, Feb. 1, 1950.

A woman with addisonian pernicious anemia developed sensitivity to liver. Folic acid controlled the hematologic situation, but symptoms of combined system disease developed. Treatment was changed to 10 mcg. of vitamin B₁₂ once a week, and improvement in the neurologic symptoms was evident within five days. After six weeks of treatment there was no subjective or objective evidence of combined system disease. At this point the supply of vitamin B₁₂ was exhausted, and it was necessary to substitute folic acid treatment. Neurologic symptoms returned under this treatment. Vitamin B₁₂ is particularly valuable in that it apparently contains factors effective against both anemia and combined system sclerosis.

An addendum states that since these observations were originally made, Lohby (et al, abstr 180) has reported that vitamin B₁₂ is not efficacious in the treatment of the megaloblastic anemias of infancy unless minute amounts of folic acid are present. This suggests that folic acid plays the role of a catalyst in this disease.

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Yonkers, N. Y.*

- 350 LEDERER, M. *Deux cas de neuroanémie traités par la vitamine B₁₂* (Two cases of neuroanemia treated with vitamin B₁₂) *Semaine d. hôp. de Paris* 26 478, Feb. 10 1950 (In Soc. Proc.)

In one woman, disturbances had developed progressively for four years, resulting in total helplessness, without a diagnosis having been made. Treatment with vitamin B₁₂ led to significant improvement in the nerve lesions, and walking was possible at the end of four months. The blood picture improved spectacularly. The second woman had had pernicious anemia for 12 years. Neurologic disturbances which had been present for two years, in spite of folic acid treatment, disappeared rapidly under the influence of vitamin B₁₂.

- 351 MUELLER, J. F., JARROLD T., HAWKINS V. R., and VILTER, R. W. *The effect of vitamin B₁₂ on the hematologic and neurologic manifestations of pernicious anemia*, Ohio State M. J. 46 225-229 March 1950.

In 7 patients vitamin B₁₂ was found to be effective in the treatment of the hematologic, neurologic and glossal manifestations of pernicious anemia. Two cases are described in detail. A table is given which describes hematologic and clinical data on all of the patients.

It was found that 1 mcg. of vitamin B₁₂ is roughly equivalent to 1 U.S.P. unit of liver extract, and 12 mcg. usually induces a satisfactory hematologic remission. In patients with combined system disease 5 mcg. every other day induces a satisfactory clinical response. It is recommended that for routine treatment of pernicious anemia, 5 mcg. of vitamin B₁₂ be given each day for 10 days and 5 mcg. every week thereafter. If neurological abnormalities are found, at least 10 mcg. daily for several months is necessary.

It is concluded that there is no evidence that vitamin B₁₂ has any more to offer a patient with pernicious anemia than liver extract. The one definite indication for

vitamin B₁₂ therapy in this disease at present is sensitivity to liver extract.

*University of Cincinnati College of Medicine
Cincinnati, Ohio*

352. GELIN G., and CANTÉGRIT M.: *Syndrôme neuro-anémique biernérien datant de six ans traité par la vitamine B₁₂ et la mécano thérapie* (Biermer's neuro-anemic syndrome of six years duration treated with vitamin B₁₂ and mechanotherapy) *Bull. Soc. méd. hôp. Paris* 66 901-904, May 1950.

353. KIMMERER, J., and HUNTER, R. B.: *Therapeutic activity of vitamin B₁₂ obtained from cultures of Streptomyces griseus* *Edinburgh M. J.* 57 2 1950 (abstr Schweiz. med. Wchnschr. 80 662, June 24, 1950)

Results obtained with vitamin B₁₂ (20 mcg. in 21 days) were similar to those obtained with liver and there were no neurologic or allergic side effects. Preexisting lesions in patients with funicular myelosis were not affected by higher doses (40 to 60 mcg.) of vitamin B₁₂.

- 354 FULD H. *Effect of vitamin B₁₂ on neuropathy in pernicious anemia treated with folic acid*, *Brit. M. J.* 2 147-148, July 15, 1950.

A 74 year old pernicious anemia patient, after developing a sensitization dermatitis from concentrated liver injections, was given folic acid, 15 mg. daily. After 21 months of this therapy she was hospitalized and diagnosed as a case of severe psychoneuropathy following folic acid treatment. She also suffered from vitamin B deficiency. Treatment with a vitamin B₁₂ preparation was begun. The initial dose was 2 cc. which is equivalent to 40 mcg. of vitamin B₁₂. It was then injected every third day for 15 days, every fifth day for 18 days longer and once a week for an additional two months. From then on she was kept on a maintenance dose of 2 cc. every two weeks. The patient had completely recovered within three months after the start of therapy.

*Birmingham General Hospital
Liverpool, England*

- 355 UNGLEY C. C.: *Vitamin B₁₂ and other dietary factors in megaloblastic anemias and in subacute combined degeneration of the cord*, *Proc. R. Soc. Med.* 43 537-541 July 1950.

356. NATARO, M. *The neurologic manifestations of pernicious anemia*, *J. Kentucky M. A.* 48 360-365 Aug. 1950.

The literature on the neurologic manifestations of pernicious anemia is reviewed (29 references) and 6 cases are reported. Results of vitamin B₁₂ therapy in these cases were similar to those obtained by adequate treatment with liver extract. Neurologic manifestations did not progress during treatment, but improvement could not be expected in all cases since most of them were of long duration. The author believes that the improvement in the neurologic manifestations under vitamin B₁₂ treatment is the result of improvement in strength and general well-being and of correction of the anemia, as well as of specific effect. The part played by the lesions in the

spinal cord is debatable. The prognosis of the neurologic manifestations of pernicious anemia depends greatly on their severity and duration prior to treatment.

Luskville, E.

- 357 SUNDGREN V: *Developments in treatment of macrocytic hyperchromic anemia: report of a case of pernicious anemia with sub-acute combined degeneration treated with vitamin B₁₂*, J. Oklahoma M. A. 43 356-358, Aug. 1950.

The author presents a review of the development of specific methods of therapy in the hyperchromic macrocytic anemias, with particular reference to pernicious anemia (10 references) and describes a patient with pernicious anemia and subacute combined degeneration. The anemia responded to refined liver extract. After 24 days of liver therapy vitamin B₁₂ became available and 10 mcg. daily was given. Thereupon the patient's neurologic signs regressed rapidly and she has been maintained on 15 mcg. of vitamin B₁₂ biweekly for a year and has shown no tendency to relapse. Of special interest is the fact that the patient's hair which was completely gray at the beginning of therapy has been gradually becoming darker. It is concluded that vitamin B₁₂ is a potent agent in the treatment of pernicious anemia, especially in cases with subacute combined degeneration.

Tulsa, Okla.

358. BORTZ, D. W., and BATTLE, J. D., Jr.: *Massive vitamin B₁₂ therapy in pernicious anemia*, Cleveland Clin. Quart. 17 166-168, 1950 (abstr. Mod. Med. 18 74 Sept. 15, 1950)

Massive doses of vitamin B₁₂ improve the neurologic manifestations of pernicious anemia more rapidly and completely than the usual small amounts. A woman who was unable to walk alone because of severe progressive posterolateral sclerosis was out of bed for several hours a week after intramuscular injection of 1,000 mcg. of vitamin B₁₂, and in 13 weeks she could walk without a cane, although numbness, tingling, and burning sensations continued. Walking ability was further improved by a second large dose, although vibratory perception did not change.

- 359 QUERIES AND MINOR NOTES: *Subacute combined degeneration of spinal cord*, J.A.M.A. 144 1224-1225 Dec. 2, 1950

A doctor's condition has been diagnosed as lateral posterior sclerosis of the spinal cord, and the writer asks about the cause, prognosis and treatment of the condition. The editor replies that the condition is usually associated with pernicious anemia but infrequently is also seen in secondary anemia and severe dietary inadequacies. It recognized early in the course and before spinal cord changes are severe, neurologic manifestations are frequently reversible. Treatment should be directed at the underlying or associated disease. In the treatment of pernicious anemia, the administration of liver extracts and/or vitamin B₁₂ will protect against the development or effect remission of subacute combined degeneration of the spinal cord. Folic acid is not effective. The provision of vitamin B complex as a part of the therapeutic regimen

may control symptoms of peripheral neuritis, which frequently accompanies this disease.

360. DOIG R. K., MOTTERDAM R., ROBERTSON E. G., and WOOD I. J.: *Early diagnosis of sub-acute combined degeneration of the cord. Value of gastric biopsy* Lancet 2 836-841, Dec. 23 1950

The authors discuss the difficulties of early diagnosis of subacute combined degeneration of the cord. They emphasize the value of gastric biopsy in determining the presence of atrophy of the gastric mucosa.

Five cases are described in detail. In one, treatment with vitamin B₁₂ was begun (results not given). In another patient, gastric biopsy showed only minimal atrophy. The patient was therefore considered a case of alcoholic peripheral neuritis and not one of pernicious anemia with subacute combined degeneration of the cord. She responded to a diet high in calories and vitamins plus parenteral vitamin B complex with considerable improvement in her mental and physical state.

Walker and Knox Hill Institute of Medical Research, and Royal Melbourne Hospital Melbourne, Australia

- 361 UNGLEY C. C., and CAMPBELL, H.: *Effect of vitamin B₁₂ in pernicious anemia and subacute combined degeneration of the cord*, Brit. M. J. 1 152-157 Jan. 27 1951.

Authors' summary: "The therapeutic value of vitamin B₁₂ in pernicious anemia was assessed and compared with that of vitamin B₁₂.

"Twenty-eight responses to single doses were observed in 24 patients. Doses were graded logarithmically—10, 20, 40, 80, and 160 µg. The increase of red blood cells in 15 days after a given dose showed the usual individual variations, but the mean of 28 responses was almost identical with the response expected from equal doses of vitamin B₁₂. The log. dose-response curve did not differ significantly from that of vitamin B₁₂. The greater consistency in response to doses of 40 µg. and over suggests that such doses would be more useful than smaller doses in future therapeutic trials.

"Twenty patients with little or no neurological involvement have been maintained for four to nine months on doses of 10 µg. every two weeks. Red blood cell or haemoglobin values were temporarily slightly subnormal in two patients; in one normality was restored with iron, and in the other without alteration of treatment. A third patient had a slightly raised M.C.V. [mean corpuscular volume]. None of the 20 patients showed neurological, lingual, alimentary or other symptoms of relapse. These results are as good, so far as those observed with similar maintenance doses of vitamin B₁₂. Such low maintenance would be dangerous, however in patients not under close observation. For routine purposes we normally give at least 60 µg. every three weeks—more if there is neurological involvement or intercurrent infection.

"The efficacy of vitamin B₁₂ against the nervous disorders of pernicious anemia was tested in six patients with subacute combined degeneration of the cord, two of whom were subjected to quantitative studies, and in 17 patients with minor neurological involvement. 7

the patients became worse and all except two were improved.

"This experiment has failed to show any difference between vitamins B₁₂ and B₁₃, but even a difference of 30% in haemopoietic potency would result in only a difference of about 5% in the average response of 28 cases; a large difference in dosage causes only a small increase in response. Later analyses based on increases of hemoglobin and of packed cell volume in 15 days also failed to demonstrate any difference in potency between the two vitamins. Although similar limitations apply to these results they greatly strengthen the conclusions based on increase of red blood cells alone. Both vitamins are of fociative against the neurological manifestations of the disease."

Royal Victoria Infirmary,
Riverside-Queen-Tyee, England

362. SCHEINBERG P: *Cerebral blood flow and metabolism in pernicious anemia*, Blood 6 213-227 March 1951

The author has measured cerebral blood flow and metabolism in 16 patients with pernicious anemia. In 7 of the patients additional studies were made during various stages of therapy. Seven of the patients received vitamin B₁₂ for periods ranging from 1 to 18 months. One patient received folic acid and liver extract and the remaining patients received liver extract or no treatment.

At the time of study the patients could be divided into two equal groups: those with severe anemia and those with moderate or no anemia, the latter groups having shown hematopoietic response to treatment. In the first group, cerebral blood flow was increased and cerebral vascular resistance decreased; in the second group, cerebral blood flow was decreased and vascular resistance increased. In both groups, cerebral oxygen and glucose consumption was decreased, as was cerebral venous oxygen tension. A good correlation was found between the mental status defects and cerebral oxygen consumption and between severity of neurologic involvement and cerebral oxygen consumption. There was no correlation between cerebrovascular resistance and cerebral oxygen consumption, nor between degree of anemia and cerebral oxygen consumption.

Specific therapy with liver extract or B₁₂ produced slight to great neurologic improvement in patients with neurologic symptoms. It also produced a moderate increase in cerebral oxygen consumption and cerebrovascular resistance. In no instance did cerebral oxygen consumption become normal.

The author discusses the disparity between the functional ability of the patients and the low values for cerebral metabolism. They conclude that pernicious anemia results in specific nervous system involvement not related to the anemia, and that this damage is at least partially irreversible in many patients.

Duke University School of Medicine,
Durham, N. C.

363. SLEPIAN A., and VAUGHN, S. L. *Nervous system manifestations in pernicious anemia: Results of treatment with liver preparations as compared with B₁₂ therapy* New York State J Med. 51 1534-1526, June 15, 1951.

On initial examination of 36 patients with pernicious anemia, 23 (78%) had neurologic abnormalities varying from peripheral neuropathy to signs and symptoms of posterolateral tract disease. Treatment was intensive both initially and in maintenance therapy; over a 21 year period it has consisted of oral liver first, then oral liver extracts, and finally parenteral extracts. One patient, in addition to these 23, after several months of treatment with folic acid alone developed spinal cord degeneration. With treatment, in the patients who had only peripheral neuritis the condition was reversed; in those without neural symptoms spinal cord disease did not develop. Those with cord involvement showed progression in their lesions, but adaptability to the neurologic disease improved in all, and under the long maintenance treatment the improvements were retained. The more extensive the disease the greater was the tendency for neurologic progression. Those faithful in their maintenance therapy progressed in their disease just as much as those with suboptimal therapy. The results suggest that prompt, vigorous treatment of neurologic and blood relapses may achieve almost the same end point. A study of these patients and others under the effects of B₁₂ will be reported later.

University of Buffalo School of Medicine, and
Buffalo General Hospital
Buffalo, N. Y.

364. CONLEY C. L., and KREVANS, J. R.: *Development of neurologic manifestations of pernicious anemia during multivitamin therapy* New England J Med. 245 529-531 Oct. 4, 1951.

Five patients are described who had neurologic symptoms characteristic of subacute combined degeneration, but whose blood showed little or no anemia. All 5 patients had taken large amounts of multivitamin preparations for long periods. It is known that the administration of folic acid can overcome the blood abnormalities in pernicious anemia, while allowing the neurologic disorder to progress. It was possible to identify the multivitamin preparation used by 4 of the 5 patients, and in each case the folic acid content was adequate to produce a hematologic remission in patients with pernicious anemia. All 5 patients were treated with intramuscular vitamin B₁₂ and in all the neurologic symptoms disappeared.

Since folic acid deficiency is quite rare, it does not seem necessary or prudent to routinely employ multivitamin preparations to which folic acid has been added. However, since these multivitamin mixtures are in widespread use, physicians must be especially careful to watch for the early manifestations of pernicious anemia. The demonstration of histamine-refractory achlorhydria in a patient with this neurologic disorder is an indication for intensive therapy with vitamin B₁₂, even though the blood and bone marrow are normal. The incorporation of small amounts of vitamin B₁₂ in multivitamin preparations does not protect patients with pernicious anemia against the development of subacute combined degeneration. Vitamin B₁₂ is poorly absorbed from the gastrointestinal tract in patients with this disease, and the vitamin must be given parenterally for optimal effect.

Johns Hopkins University and Hospital
Baltimore, Md.

- 365 CHODOS R. B., and ROSS, J. F.: *The effects of combined folic acid and liver extract therapy* Blood 6 1213-1233, Dec. 1951

It has been found that folic acid therapy sometimes exerts a deleterious effect on the central nervous system of patients, especially those with pernicious anemia. The present study concerns patients with pernicious anemia treated initially with folic acid and later with a combination of folic acid and vitamin B₁₂ or liver extract.

Authors summary "Folic acid, when administered alone, did not prevent the development or progression of subacute combined degeneration in 12 of 22 patients receiving this agent for from twelve to twenty five months. One patient with total gastrectomy and a macrocytic anemia developed subacute combined degeneration after five months of folic acid therapy. Folic acid therapy did not produce neurologic disease in patients with iron deficiency anemia who had free gastric hydrochloric acid in their gastric secretions and presumably sufficient intrinsic factor. It did not influence response to ferrous sulfate therapy. Neurologic disease did not develop in 6 pernicious anemia patients treated with folic acid and liver extract for three and one-half to thirty-nine months. In 10 pernicious anemia patients with good nutrition, neurologic relapses did not progress when liver extract or vitamin B₁₂ therapy was instituted, even though folic acid therapy was continued. In 2 patients with abnormal nutrition and complicating organic abnormalities, nervous system disease progressed after institution of liver extract therapy. The hematologic status of patients with pernicious anemia is not maintained in a more satisfactory state by supplementation of liver extract or vitamin B₁₂ therapy with folic acid."

These findings are explained by the theory "that the hematologic and neurologic manifestations of pernicious anemia and other macrocytic anemias associated with gastro-intestinal tract pathology and inadequate nutrition are due to a deficiency of more than one substance. The administration of folic acid may improve the hematologic status but induce a deficiency of another substance or substances, e.g., vitamin B₁₂, which are essential for the maintenance of a normal blood picture and the integrity of the central nervous system. This deficiency will eventually result in the development of a suboptimal blood picture or subacute combined degeneration of the spinal cord, or both."

The authors recommend that patients with sprue, nutritional macrocytic anemia and other macrocytic anemias associated with gastrointestinal tract pathology who are treated with folic acid should also be given supplemental liver extract or vitamin B₁₂ to insure against the development of nervous system disease.

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Boston University School of Medicine
Boston, Mass.
Cordell F. H. Hospital
Framingham, Mass.*

366. SAMSON D. C., SWISHER, S. N., CHRISTIAN R. M., and ENGEL, C. L.: *Cerebral metabolic disturbance and delirium in pernicious anemia: clinical and electroencephalographic studies* A.M.A. Arch. Int. Med. 90 4-14, July 1952.

In 13 of 14 patients with pernicious anemia in hematologic relapse, tests showed a reduction in awareness justifying the diagnosis of delirium. This was presumed to be the result of a disturbance in cerebral metabolism and was accompanied with more or less irreversible electroencephalographic changes. Under treatment with vitamin B₁₂, the delirium regressed and the electroencephalogram began to show return toward normal at about the time when the reticulocyte response began, which suggests that the cerebral defect is primary rather than secondary to the anemia. The mental improvement was primarily on the level of awareness, and not on the more personal aspects of behavior. A patient with paranoid trends improved in awareness but showed no change in his paranoid behavior. The authors state that there is no reason, from their studies, to anticipate that vitamin B₁₂ or related antianemic factors will affect other psychiatric disorders or even delirium in patients who do not have pernicious anemia.

*Strong Memorial Hospital,
Rochester Municipal Hospital, and
University of Rochester School of Medicine and Dentistry
Rochester, N. Y.*

- 367 BASTRUP MADSEN P.: *Cytology of bone marrow in non-anemic funicular myelopathy (subacute combined degeneration of spinal cord)* Ugesk. L. laeger 114 1853- Dec. 25, 1952 (abstr. J.A.M.A. 152 288, May 16, 1953)

"Bastrup-Madsen presents further clinical evidence that the spinal cord lesions of pernicious anemia can occur as an independent neurological affection that leads to disability or continues for years without the development of a regular pernicious anemia. Ten cases of subacute combined degeneration of the spinal cord with histamine-fast achylia are described. In six cases the hemoglobin and erythrocyte values were normal, and in four cases there was a slight reduction in the erythrocyte count. Examinations of the bone marrow revealed slight maturation disturbances in the erythropoiesis in seven cases. In all cases there were perniciousiform disturbances in maturation of granulocytes, testifying to a lack of antipernicious anemia factor as the basis for the myelopathy. In five cases examinations of the bone marrow after two weeks of antipernicious anemia treatment showed normal bone marrow. In two additional cases there was a slight rise in the erythrocytes during treatment. In five cases some improvement in the neurological symptoms occurred during the specific treatment. In nine cases the disorder had persisted for from 2 to 20 years, and in seven cases more or less disability had developed before the start of antipernicious anemia treatment. In these cases specific antipernicious anemia treatment must be instituted without waiting for the development of anemia. Examination of the bone marrow may be a valuable diagnostic aid even though the peripheral blood shows normal conditions. The same substances, liver and stomach preparations, and vitamin B₁₂, seem to be important for both the central nervous system and the bone marrow."

368. EARL, C. J., EL HAWARY M. F. S., THOMPSON R. H. S., and WEBSTER, C. R.: *Blood pyruvate levels in subacute combined degeneration of cord effect of vitamin B₁₂ therapy* Lancet 1: 115-11 Jan. 17 1953.

Raised blood pyruvate levels were found in 3 patients with untreated subacute combined degeneration of the cord. Parenteral treatment with 140 to 200 mcg. of vitamin B₁₂ per week in divided doses led to a rapid return to normal of these levels.

The neurologic symptoms and blood counts improved in all 3 patients, but this occurred more slowly than did the normalization of pyruvate blood levels.

The authors come to the tentative conclusion that "in subacute combined degeneration of the cord there is a defect of pyruvate metabolism which can be rectified by treatment with vitamin B₁₂."

Gay's Hospital Medical School
London, England

369 HOWARD R., in discussion on Hammarsten, J. F. *Clinicopathological Conference, Minneapolis Veterans Hospital, Presentation of case, Journal Lancet* 73 101 102, 118, March 1953.

Dr. Howard reports having seen a patient with pernicious anemia who had an exacerbation of associated neurologic complications after taking a mail-order preparation containing B₁₂ and folic acid.

University of Minnesota
Minneapolis, Minn.

DIABETES MELLITUS

370. SCOTT R. W., SANCETTA, S. M., and AYRES, P. R.: *A preliminary note on the use of vitamin B₁₂ in the management of the neurologic manifestations of diabetes mellitus*, *Tr. A. Am. Physicians* 63 232-240, 1950.

371 SANCETTA, S. M., AYRES, P. R., and SCOTT R. W. *The use of vitamin B₁₂ in the management of the neurologic manifestations of diabetes mellitus, with notes on the administration of massive doses*, *Ann. Int. Med.* 35 1023-1048, Nov. 1951.

Since vitamin B₁₂ has been found beneficial in the neurologic changes in patients with pernicious anemia, the authors have used it, together with insulin and dietary control, in 12 diabetic patients with neurologic disturbances. Case histories are given. Three patients are regarded as having complete neurologic remission, 1 had a partial relapse during treatment, followed by complete remission, 3 are considered in almost complete remission, 2 as improved, and 2 as questionably improved. The response in such patients varies in accordance with the extent of vascular damage and the reversibility of injury to nerve cells. Damage to nerve tissue as a result of arteriosclerosis of the vasa nervorum is presumably irreversible. It is doubtful that intrinsic spinal cord lesions can be reversed. Improvement is not to be expected unless effective regulation of diabetes with insulin and diet is instituted.

Exact dosage of vitamin B₁₂ has not been established. A flexible dosage of 15 to 50 mcg. daily for the first 7 to 14 days is recommended, followed by a maintenance dosage of 15 to 50 mcg. once or twice a week. Frequency of administration appears to be more important than the amount of individual doses in achieving and maintaining a neurologic remission. Crystalline vitamin B₁₂ given in-

transcutaneously or subcutaneously was used except in one patient who was treated exclusively with streptomycin broth concentrate B₁₂.

Concentrations of 900 and 1,000 mcg. of vitamin B₁₂ per cc. were used in some patients. In one of them the administration of 900 mcg. gave results similar to those obtained with 15 mcg. Two whose residual symptoms consisted of occasional pain in the feet at night were not relieved by daily injections of 900 mcg. each for five days. One patient, an alcoholic who showed no improvement until satisfactory control of the blood sugar had been attained, was refractory to small doses of vitamin B₁₂ but responded dramatically to 1,000 mcg. doses 10 such doses were given on alternate days. A second course was given later. About a month after vitamin B₁₂ had been discontinued, three doses of 1,000 mcg. each were given at weekly intervals, followed by 15 mcg. once a week, which keeps the patient free from pain except for dull aches occurring on the sixth day after each injection. It is doubtful if the enormous doses used in this case were necessary. The authors conclude that massive dosage of vitamin B₁₂ may be indicated in the exceptional patient showing severe manifestations, but it is doubtful that improvement can be attained more quickly and completely than with the usual small doses. The improvement of these patients appears to be the result of the action of vitamin B₁₂ on the deranged intrinsic nerve metabolism, insofar as the latter is not partially caused by obliterative disease of the vasa nervorum. The mode of action is not known.

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Cleveland, Ohio

372. BEIDLEMAN B. *Critical vitamin deficiencies in patients with diabetes mellitus*, *J. Clin. Nutrition* 1 119-123, Jan. 1953.

Patients with diabetes are often given diets low in calories or fat which do not supply enough vitamins. Perifollicular hyperkeratosis and dry skin may result from vitamin A deficiency. Theoretically vitamin D deficiency might result from these diets, but the author has not encountered signs of it in diabetes.

Peripheral neuritis, neurasthenia, anorexia, burning sensations in the mouth, soreness and redness of the tongue, cheilitis, tenderness along the major peripheral nerve trunks, and absence of tendo Achillis reflexes are the most frequently encountered signs of vitamin B deficiencies. Thiamine alone may clear the signs and symptoms of peripheral neuritis, and lesions of the skin and mucous membranes may respond to thiamine, riboflavin, or niacin, or even pyridoxine or biotin. Although the major deficiency may be of a single vitamin, deficiencies of the others may account for some of the symptoms. Thiamine with riboflavin and niacin has been suggested for the treatment of diabetic coma, but the author has not found that omission or addition of large doses of this vitamin complex influences the rate of recovery from coma.

Ascorbic acid and rutin have been suggested for treatment and prevention of diabetic retinopathy because of their effect on the integrity of the capillary wall. The author is prescribing three tablets a day each containing 100 mg. of ascorbic acid and 20 mg. of rutin, but has seen no reversal of retinopathy although its progression may have been slowed.

In patients whose diabetes has not been well controlled hepatic enlargement which responds to choline may occur

True diabetic neuropathy is manifested by signs and symptoms similar to those seen in patients with the combined system disease of pernicious anemia. Many patients with mild to moderate neuropathic symptoms have been promptly and lastingly relieved by daily administration of 30 mcg. of vitamin B₁₂ for three to five days. Some patients require a maintenance dose of this vitamin to prevent recurrences. In 1 patient ataxia, nocturnal diarrhea, peripheral neuritic symptoms, and a left sixth cranial nerve palsy completely disappeared within a month of vitamin B₁₂ therapy. In some patients severe neuropathies appear to have reached an irreversible stage, and vitamin B₁₂ has only a slight effect or none at all even in large doses over prolonged periods.

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Jefferson Medical College
Philadelphia, Pa.
(see also)
Medical Center Clinic
Pensacola, Fla.*

373. MENOF P. *New treatment for polyneuritis*, South African M. J. 24 715- Aug. 26, 1950 (abstr J.A.M.A. 144 1521 Dec. 23, 1950)

Vitamin B₁₂ was administered to patients with alcoholic and diabetic polyneuritis. The doses used were considerably larger than those recommended for pernicious anemia. The author began with 60 mcg. [and finally gave as much as 210 mcg.] in one injection. The best result was achieved with this large dose. The treatment was given to 11 patients with encouraging results. Apart from a little localized tenderness due to the large size of the intramuscular injection (7 cc.) no undesirable side effects were noted. The author feels that vitamin B₁₂ might be effective in other forms of polyneuritis and in other nervous disorders associated with alcoholism, e.g., delirium tremens, Korsakoff's psychosis, hemorrhagic polioencephalitis, and alcoholic hallucinosis. Vitamin B₁₂ might also be tried in such diseases as progressive muscular atrophy

374. MENOF P. *Role of vitamin B₁₂ in polyneuritis fatty degeneration and pernicious anaemia*, South African M. J. 25 894-999 June 9 1951 (abstr J.A.M.A. 147 791, Oct. 20 1951)

"Menof recently called attention to the remarkable curative action of vitamin B₁₂ in the polyneuritis associated with chronic alcoholism and diabetes mellitus. He reported a dramatic response in nine of 11 cases. Since that time he has treated 19 similar patients (seven alcoholic, four diabetic, and one diabetic and alcoholic). The response has been uniformly satisfactory. The tenderness and volume of the liver receded almost as rapidly as the neurological disturbances. The author feels that vitamin B₁₂ should be used wherever fatty degeneration is encountered. It is also indicated in any condition where demyelination or defective red blood cell structure is believed to be the underlying pathological factor. Reasons are given why pernicious (Addison's) anemia should now be regarded as a disorder of lipid metabolism rather

than as a disease of the blood. Evidence is adduced to show that the action of vitamin B₁₂ is to synthesize the various chemical substances that make up certain phospholipids or cerebroside or both."

NEUROPATHIES IN GENERAL

375. BEAN W B., FRANKLIN M., and SACHS A. L.: *Preliminary note on the effect of vitamin B₁₂ on the painful aspects of nutritional neuropathy* Proc. Central Soc. Clin. Research 22 10-11, Nov 1949 (also abstr Mod. Med. 18 108, Feb. 1 1950)

Because it has been questioned whether vitamin B₁₂ actually is the factor concerned in the neuropathies of beriberi, and because vitamin B₁₂ has successfully controlled combined system disease in pernicious anemia, the authors tried vitamin B₁₂ in 14 patients with painful neurologic disorders of other origins. In 2 patients the neurologic symptoms were associated with nutritional deficiency and in 1 with chronic alcoholism and diabetes. In 11 they apparently were unrelated to nutritional factors. The first three patients had dramatic relief of pain following one intramuscular injection of 15 mcg. of vitamin B₁₂. In the remaining 11 patients, who were suffering variously from brachial neuritis, multiple sclerosis, diabetic neuritis, tabes, trigeminal neuralgia, monocytic leukemia, ruptured disk and alcoholic psychosis, no relief of pain was obtained by this treatment.

376. BEAN W B., FRANKLIN M., and SACHS A. L. *An effect of vitamin B₁₂ on pain in nutritional neuropathy* Am. J. M. Sc. 220 431-434, Oct. 1950 (also cited in Nutr Rev 9 207-208, July 1951)

The authors describe 3 patients with painful nutritional neuropathy in whom the pain disappeared within 30 to 60 minutes after the injection of 15 mcg. of vitamin B₁₂ concentrate. Adequate control studies were made. The patients subsequently showed improvement on a good diet supplemented with yeast, vitamin B complex tablets, riboflavin and niacin.

Twenty five patients with pain caused by a miscellaneous group of neurologic disturbances did not respond to the administration of vitamin B₁₂ concentrate.

The mechanism of action of B₁₂ is unknown. Its effect, however is believed to be direct. Moreover an experiment is cited which shows that the results obtained are not an effect of analgesia.

In an addendum, a case is cited in which the patient improved after the injection of 6 mcg. but not of 3 mcg. of vitamin B₁₂. Another patient who was moribund did not respond to 80 mcg. of B₁₂.

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University Hospitals
Iowa City, Iowa*

377. SPILLANE, J D. *Vitamin deficiencies and the nervous system*, Practitioner 2 143- 1950 (abstr Schweiz. med. Wchnschr 80 926, Aug 26, 1950)

In animal experiments, deficiencies of vitamins A and E and B complex factors lead to neurologic disturbances. In man, only the members of the B complex are of importance to the nervous system. Thiamine is necessary for cellular respiration, and riboflavin, nicotinic acid, pyridoxine and pantothenic acid are necessary for proper functioning of the nervous system. It is pointed out that polyneuritic symptoms frequently occur in patients with gastrointestinal diseases and in alcoholics whose nutrition is inadequate. It is possible that spinal cord degeneration is caused by vitamin B₁₂ deficiency. This condition is often associated with pernicious anemia and it improves when the anemia improves as a result of vitamin B₁₂ therapy.

NEUROLOGIC DISEASE

AMYTROPHIC LATERAL SCLEROSIS

- 379 SPIES T. D., and STONE, R. E.: *Some observations on patients with amyotrophic lateral sclerosis*. South. M. J. 42: 410-411, May 1949

Five cases of progressive muscular atrophy due to spinal cord degeneration were selected for treatment with vitamin B₁₂. Four of these were diagnosed as amyotrophic lateral sclerosis. In the fifth, the symptoms were similar except that no involvement of the lateral column was evident. These patients had been effected for one to four years, and many kinds of treatment had failed. In all 5, the disease was progressive. In each case there was great subjective improvement, with diminution in muscle cramps and stiffness, following the administration of vitamin B₁₂. While there were no measurable objective changes, there was no progression of the disease process during several weeks of observation, and treatment will be continued to see whether the disease process can be halted by frequent injections of the vitamin. The patient whose case is summarized in this article was given 10 mcg. of B₁₂ three times a week.

Birmingham, Ala.

380. SPIES, T. D., HAUSER, E., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., ARAMBURU, T., and STONE, R. E.: *Observations sur l'action de la vitamine B₁₂ sur des sujets atteints de sclérose combinée, associée à l'anémie pernicieuse la sclérose en plaque la sclérose latérale amyotrophique et les séquelles de la poliomyélite* (Observations on the effect of vitamin B₁₂ on patients with combined system disease associated with pernicious anemia, multiple sclerosis, amyotrophic lateral sclerosis, and residual changes of poliomyelitis). Internat. Zschr. f. Vitaminforsch. 21: 347-353, 1949

The administration of adequate doses of vitamin B₁₂ ameliorated the symptoms of combined sclerosis, especially the acute symptoms. In all but 1 of the 60 cases with pernicious anemia and associated combined system disease the clinical and hematologic response to vitamin B₁₂ was good. This one patient relapsed while under treatment: thiamine was ineffective but liver extracts brought about a response. In multiple sclerosis, amyotrophic lateral sclerosis and residual changes of poliomyelitis,

378. HARVIER, P., CLAISSE, R., and CARAMANIAN M. K.: *Syndrôme neuroanémique grave non bier merien. Guérison spectaculaire par la vitamine B₁₂* (Spectacular cure with vitamin B₁₂ of a severe neuroanemic syndrome—not Biermer's anemia). Semaine d. hôp. de Paris 26: 3757 Sept. 25, 1950 (In Soc. Proc.)

A severe neuroanemic syndrome occurring in the course of serious posthemorrhagic anemia (not pernicious) was almost completely cured by eight days' treatment with vitamin B₁₂. The static and bedridden patient recovered his normal aspect and activity within a few days.

B₁₂ brought about some subjective but little objective improvement. The administration of 15 mcg. of vitamin B₁₂ five times a day in 40 cases of acute poliomyelitis gave no amelioration except a slight subjective amelioration in 4 cases. The authors consider B₁₂ the most effective substance at present available for use in Biermer's macrocytic, and nutritional anemia, and in sprue.

MULTIPLE SCLEROSIS

381. LEREBoullet J., PLUVINAGE, R., and COTY, R.: *Vitamine B₁₂ et sclérose en plaques* (Vitamin B₁₂ and multiple sclerosis). Presse, méd. 58: 230, March 4, 1950 (In Soc. Proc.) J.A.M.A. 143: 1272, Aug. 5, 1950 (In Foreign Letters)

Seven patients with multiple sclerosis characterized by extreme sensitivity in addition to the usual signs of this disease were treated with large doses of vitamin B₁₂. The sensitivity disappeared and there was great functional amelioration. A more prolonged study is needed to determine whether the pyramidal pain is likewise affected. In discussion, Peranté emphasized the interest of this communication which may contribute to the explanation of the mechanism of action of vitamin B₁₂ on the neurologic-anemic syndrome. Significant amelioration of neurologic signs is often observed before the hematologic disturbance is completely ameliorated.

382. JONEZ, H. D.: *Certain vascular effects of histamine and d-tubocurarine in multiple sclerosis. Part III* Ann. Allergy 8: 188-193, March-April, 1950.

In a series of 254 patients treated by intravenous injections of histamine, many received adjunctive treatment with d-tubocurarine and vitamin B₁₂.

"The allergy theory of multiple sclerosis etiology and therapy does not conflict with or displace the therapy calling for the prevention of vasospasm and continued vasodilatation."

314

a review
Aug.

treatment of multiple
J.A.M.A. 143

In a review article on the drugs which have been used in the treatment of multiple sclerosis, the author cites an article of his own, soon to be published, on the treatment of multiple sclerosis with vitamin B₁₂.

334. SIMSON G., HERFORD A., KRIM, M., and MEYER, L. M.: *Effects of vitamin B₁₂ in multiple sclerosis*, Proc. Soc. Exper. Biol. & Med. 75: 721 Dec. 1950.

Five patients with multiple sclerosis and 1 patient with amyotrophic lateral sclerosis failed to respond clinically or hematologically to daily intramuscular injections of 1,050 mcg. of vitamin B₁₂. Treatment was continued for three weeks in a case of multiple sclerosis of one month's duration the other patients, who had all been ill for years, were treated for four months (1 case) or five months.

Calcutta: H. S. Ghosh, 11, Upper Circular Road, N. 7

335. BOOTH, C. B., LAWYER, T. J., JR., and VON STORCH, T. J. C.: *Vitamin B₁₂ in the treatment of multiple sclerosis*, J.A.M.A. 147: 894, Oct. 27 1951 (In Correspondence)

Because of conflicting reports in the literature regarding the effects of vitamin B₁₂ in multiple sclerosis, the writers made a study of 24 patients with a justifiable diagnosis of multiple sclerosis who were treated with vitamin B₁₂. Six of the patients were treated in a hospital and 18 as outpatients. Six patients received 100 mcg. of vitamin B₁₂ intramuscularly every other day for three months, and 5 of these received 1,000 mcg. of the vitamin every other day for an additional three months. Fifteen patients received 100 mcg. of vitamin B₁₂ three times a week for three months, 2 received 1,000 mcg. of vitamin B₁₂ three times a week for three months, and 3 have received 1,000 mcg. of the vitamin three times a week for two months, and therapy is continuing.

About one-fourth of the patients noted an increase in appetite and a sense of well being while receiving vitamin B₁₂ therapy. There was, however, no objective evidence of neurologic improvement aside from the type of fluctuation which is characteristic of the natural course of the disease. After cessation of therapy two patients claimed that they felt much stronger and were definitely benefited by vitamin B₁₂. They were therefore given placebo for two months, and reported the same dramatic improvement with the placebo as they claimed for vitamin B₁₂. It is concluded that vitamin B₁₂ is not effective in the treatment of multiple sclerosis.

New York: H. S. Ghosh, 11, Upper Circular Road, N. 7

336. LEREBULLE J., and PLUVINAGE, R.: *Use of vitamin B₁₂ in neurology* J.A.M.A. 148: 667 Feb. 23, 1952 (In Foreign Letters)

" Drs. J. Lereboullet and R. Pluvinaige reported on the use of vitamin B₁₂ in the treatment of 74 patients with neurological disease. In 10 of 43 patients with multiple sclerosis, there were no positive reactions. In 16 patients with advanced cases, there were 4 instances of only subjective improvement. In 17 patients with cases of average intensity there was noticeable improvement

and, in 4 instances, the patients were able to resume their normal occupations after years of illness and failure of all previous treatment. In eight patients with spastic paraplegia, including two cases of unknown origin and two cases that occurred after serious accidents, functional improvement was observed. In six out of eight patients with spinocerebellar syndromes of the atromegaly type, functional improvement and a diminution of ataxia were noted. Four patients, formerly invalids, were able to resume their former activities. In two children with cerebellar atrophy vitamin B₁₂ brought about an ambulatory state and a considerable diminution of objective cerebellar signs. In seven patients with polyneuropathy, including three with Korsakoff's psychosis, results were spectacular. The three patients with Korsakoff psychosis were cured within one week. The authors believe that vitamin B₁₂ has a positive action on early polyneuropathy but has no effect on old lesions. In regard to the mechanism of action of vitamin B₁₂, the authors consider two hypotheses. The first is that vitamin B₁₂ acts on myelinic elements, especially in neurotrophic syndromes and in certain cases of multiple sclerosis, in which demyelination, with comparative conservation of axons, occurs. The second hypothesis assumes an occasional direct stimulation of functions of the elements not destroyed."

HERPES ZOSTER

337. JOLLES, K. E.: *Herpes zoster treated with vitamin B₁₂*, Brit. M. J. 1: 959 April 22, 1950 (In Correspondence)

Six cases of herpes zoster were treated with vitamin B₁₂ concentrate with uniformly excellent results. The stages of the disease at which therapy was instituted varied from early intense erythema before vesiculation to a case of heavily secondarily infected herpes zoster with considerable underlying cellulitis. The treatment has not yet been tried in cases of postherpetic neuralgia.

Vitamin B₁₂ was given in intramuscular injections of 2 cc. of the concentrate on the first, second, and fourth days of treatment. Injection was absolutely painless. Considerable clinical and objective improvement followed the first injection, new lesions ceased to appear and by the end of the first week all local signs of the disease were gone.

West Bromwich, England

338. HOWARD C.: *Liver extract for herpetic pain*, Lancet 2: 242, Aug. 2, 1952.

The writer describes 4 patients with herpes to substantiate his claim that liver extract helps relieve herpetic pain.

"In 3 of them the pain disappeared in twenty four hours, never to return. In the 4th the pain, which was agonizing and involved the area supplied by the left brachial plexus, disappeared, but was succeeded by intense itching. In each case 'Campolon' 2 ml. was injected intramuscularly and it seemed to me that the change of infection in the last case might be due to an insufficient amount of some constituent in the liver extract.

"I therefore quite empirically gave an injection vitamin B₁₂ (Cytamen 2 ml.) and the itching van

overnight. When I last saw the patient a week ago he had been free from symptoms, except slight numbness, for a month."

London, England

- 389 LEITCH, G. B. *Vitamin B₁₂ in massive dosage for herpetic lesions: a preliminary report*, Northwest Med. 52: 291-292, April 1953.

Because of favorable reports of the effect of vitamin B₁₂ on neurologic conditions in pernicious anemia and on trigeminal neuralgia, the author administered it to a patient for severe pain from herpes zoster which had resisted various types of treatment. The vitamin was used in the strength employed in treating trigeminal neuralgia—1,000 mcg. in a volume of 10 cc., half of which was injected into each buttock. This was repeated on the next day by the third day a preparation containing 1,000 mcg. per cc. was available and was substituted for the less concentrated solution. Herpetic pain disappeared within 24 hours after administration of the first injection, and the lesions began to heal within 48 hours. After five days the dosage was reduced to 500 mcg. on alternate days. After the second 500 mcg. injection the patient left the hospital one more 500 mcg. dose and two of 100 mcg. each, on alternate days, concluded the treatment. The usual peripheral neuritis was so mild that the patient refused treatment. In 4 other cases, which were less severe, vitamin B₁₂ was equally successful. In only one of these cases was mild sedation for peripheral neuritis required. In a single case of herpes simplex involving the lips, the patient was able to preach after three injections of 1,000 mcg. each although crusts remained. There were no unfavorable reactions to daily injections of 1,000 mcg. of vitamin B₁₂.

The author considers further trial of vitamin B₁₂, alone or with other remedies such as thiamine hydrochloride, indicated in herpes. The only reference which he found in the literature was a report by Heyblon (*Méd. française* 11: 102, May 1951) of recovery from herpes zoster in two to six days in 8 of 11 patients treated with vitamin B₁₂; the recommended dosage was 30 mcg. daily for eight days.

Portland, Oregon

OTHER NEUROLOGIC DISORDERS

- 390 ARKLESS, H. A. *Reactions to vitamin B₁₂*, J.A.M.A. 144: 1586, Dec. 30, 1950 (in Correspondence)

Subcutaneous injections of vitamin B₁₂ concentrate were given to a 47 year old woman who had multiple radicular neuralgias of long duration. The first four injections were without reaction; the fifth and sixth were followed by swelling and pain in both arms. No general effects were noted by the patient; the swelling subsequently disappeared.

555 So. Commonwealth St.
Philadelphia, Pa.

- 391 JAMIESON S. R.: *Tuberculous meningitis treatment with streptomycin and para-aminosalicylic acid*, Brit. M. J. 1: 83-85, Jan. 12, 1952.

In addition to intramuscular and intrathecal streptomycin combined with oral PAS all patients showing muscular wasting were given one tablet of a proprietary vitamin B₁₂ preparation daily. These patients increased in weight, had improved appetites and became more active.

Croft's Hill Hospital
Croydon, East Yorkshire
England

- 392 FIELDS W. S., and HOFF H. E. *Relief of pain in trigeminal neuralgia by crystalline vitamin B₁₂*, Neurology 2: 181, 1952.

Thirteen patients with trigeminal neuralgia experienced remarkable relief after treatment with large intramuscular doses of vitamin B₁₂. Six patients received 1,000 mcg. of vitamin B₁₂ biweekly or triweekly for four to eight weeks. The lancinating pain gave way to a burning parasthesia which disappeared later. Supplies of the drug were not available for 2 of the patients for about one week shortly after the typical attacks stopped. In both, the lancinating pain returned but was relieved after the first of six daily 1,000 mcg. injections. The last 7 patients were treated with 10 daily doses of 1,000 mcg. of vitamin B₁₂.

Nine of the patients had not had previous surgical intervention, and these experienced prompt and complete remissions. Relief from pain was delayed in the 4 cases where surgery or nerve block had been resorted to, but "ultimately results appeared to be equally (as) good" as for the other 9 patients.

Baylor University College of Medicine
Houston, Texas

- 393 PHILLIPS, G. B., VICTOR, M., ADAMS, R. D., and DAVIDSON, C. S. *A study of the nutritional defect in Wernicke's syndrome: the effect of a purified diet, thiamine, and other vitamins on the clinical manifestations*, J. Clin. Investigation 31: 859-871, Oct. 1952.

Authors' summary and conclusions: "1. Nine patients with Wernicke's syndrome, characterized by ophthalmoplegia, nystagmus, ataxia, and mental disturbances were given a purified diet composed solely of glucose and minerals. Specific vitamins were added after appropriate intervals of observation.

"2. Prior to the administration of thiamine, there was no improvement in any of the signs. More specifically despite alcohol withdrawal, bed rest, and the addition of other vitamins (niacin, calcium pantothenate, pyridoxine, folic acid, ascorbic acid, riboflavin, B₁₂), the ophthalmoplegia progressed and the ataxia remained unchanged, while the nystagmus decreased only in association with an increase in ocular paralysis.

"3. When thiamine only was added in the purified diet, the ophthalmoplegia cleared considerably in from one and a quarter to six hours; diminution in nystagmus and ataxia also occurred in some of the patients, but the change was more gradual; there was improvement in mental disturbance, but it was minimal in degree and consisted of increased attentiveness and capacity to maintain a conversation and greater ease of confabulation.

"In view of these observations, there seems to be little doubt that the ophthalmoplegia of Wernicke's syndrome is related to a specific lack of thiamine. The nystagmus and ataxia also appear to be related to thiamine deficiency but the evidence is less conclusive. No definite conclusions can be drawn regarding the relationship of the mental disturbances to the deprivation of thiamine or other vitamins."

Thurston Memorial Laboratory
Massachusetts General Hospital,
Boston City Hospital, and
Harvard Medical School
Boston, Mass.

- 394 FRIEDLANDER, W. J.: *Meralgia paresthetica*
U S Armed Forces M. J. 3 1857 1862, Dec. 1952.

Meralgia paresthetica occurring in 14 soldiers is described. The disease, which is a neuropathy of the lateral femoral cutaneous nerve, is not uncommon, but is often misdiagnosed. Three of the patients were given 120 mcg. of vitamin B₁₂ intramuscularly daily for two weeks, but this treatment had no beneficial effect. The disease usually disappears spontaneously but sometimes nerve resection or procaine nerve block is necessary.

U S Army Hospital
Fort Bragg, N. C.

ANIMAL STUDIES

395. HOGAN, A. G., O'DELL, B. L., and WHITLEY, J. R.: *Maternal nutrition and hydrocephalus in newborn rats*, Proc. Soc. Exper. Biol. & Med. 74 293-296, June 1950.

The incidence of hydrocephalus in the young of female rats given an experimental diet containing soybean oil meal and a vitamin mixture that included all recognized vitamins except ascorbic acid, niacin, folic acid and B₁₂ was less than 1 per cent. When folic acid deficiency was produced by adding a folic acid antagonist, crude methyl-folic acid, to the diet, the incidence of hydrocephalus rose to 20 per cent. There was a long delay in the appearance of hydrocephalic young when the females were kept on a casein diet before transfer to the soybean oil meal diet containing a folic acid antagonist. The casein diet did not contain folic acid, and the delay in appearance of hydrocephalus may indicate that another nutrient is involved, possibly vitamin B₁₂ since the stock diet undoubtedly contains it and the casein used contained 1.8 mcg. per 100 cc., while soybean oil meal is known to be a poor source.

University of Missouri
Columbia, Mo.

396. O'DELL, B. L., WHITLEY, J. R., and HOGAN, A. G.: *Vitamin B₁₂, a factor in prevention of hydrocephalus in infant rats*, Proc. Soc. Exper. Biol. & Med. 76 349-353, Feb. 1951.

Rats that had been maintained from weaning to maturity on a diet deficient in vitamin B₁₂ were then given the same diet with supplements of a folic acid antagonist. Twenty-three per cent of their young were hydrocephalic and only 17 per cent were weaned at the age of 4 weeks. Rats given the same experimental diet without previous depletion produced 15 per cent hydrocephalic young and weaned 21 per cent; rats without previous depletion that were given a vitamin B₁₂ concentrate (animal protein factor supplement) which supplied 2.2 mcg. of vitamin B₁₂ per 100 Gm. of diet produced no hydrocephalic young and weaned 81 per cent.

Rats that had consistently produced hydrocephalic young were given 1 mcg. of crystalline vitamin B₁₂ each week parenterally. No hydrocephalic young were produced by rats in which vitamin B₁₂ administration had been started five weeks before parturition or on the seventh day of gestation, but in the latter group only 14 per cent of the young were weaned. When vitamin B₁₂ was not given until the sixteenth day of gestation, 50 per cent of the young were hydrocephalic.

Supplements of folic acid alone did not prevent hydrocephalus in the young. The soybean oil meal used in the basal diet in these experiments contains some vitamin B₁₂ and it is not certain whether vitamin B₁₂ alone is effective or whether both folic acid and vitamin B₁₂ are required.

University of Missouri
Columbia, Mo.

397. ALEXANDER, W. F., and BACKLAR, B.: *Ascleic acid changes in rat nerve tissue after parenteral administration of vitamin B₁₂*, Proc. Soc. Exper. Biol. & Med. 78 181-184, Oct. 1951.

Authors summary "These preliminary results support the contention that vit. B₁₂ is related to the genesis of ribonucleoproteins in rat tissues. Weanling and immature rats were placed in 2 dietary groups, chow and basal, the latter dietary group deficient in vit. B₁₂, folic acid and PARA. One-half of each group received parenteral B₁₂ every day. Liver, spinal cord and cervical sympathetic ganglia were examined histologically for changes in pentoses and deoxypentoses nucleic acids. These 3 tissues from animals which received vit. B₁₂ showed more DNA than the controls. No consistent observable difference was seen in spinal cord and cervical sympathetic ganglia for the DNA as visualized by the Feulgen stain. There was a slight increase in the intensity of the Feulgen stain in hepatic cell nuclei from rats which had received vit. B₁₂ over the controls."

St. Louis University School of Medicine
St. Louis, Mo.

Gastrointestinal

SPRUE

398. SPIES, T. D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and CULVER, B.: *Observations on the hemopoietic response of persons with tropical sprue to vitamin B₁₂*. South. M. J. 41: 523-525, June 1948.

Two patients with tropical sprue in relapse were each given a single intramuscular injection of 8 mcg. crystalline vitamin B₁₂. Each responded clinically and hematologically. By the third day the patients voluntarily said that they felt stronger and by the sixth day soreness of the mouth and tongue had completely disappeared, appetite had returned, and the stools had decreased in volume. Both patients showed a rise in reticulocytes which reached a peak on the eighth day. This was followed by an increase in red blood cells and hemoglobin and, in 1 case, by a rise in white blood cells.

Northwestern University
Chicago, Ill.
Gen. Collins Garcia Hospital
Pinar, Cuba

399. SUAREZ, R. M., and SPIES, T. D.: *The effectiveness of vitamin B₁₂ in the treatment of tropical sprue*. Bol. Asoc. med. de Puerto Rico 40: 199-209 Aug. 1948.

400. SPIES, T. D., and SUAREZ, R. M.: *Response of tropical sprue to vitamin B₁₂*. Blood 3: 1213-1220, Nov. 1948.

The response of 5 patients with tropical sprue to vitamin B₁₂ given intramuscularly is described. Two patients received three single doses of 10 to 25 mcg. at various time intervals, 1 patient received two single doses of 25 mcg., 1 a single dose of 20 mcg. and 1 a single dose of 4 mcg. An injection of 10 to 25 mcg. always produced a reticulocytosis, beginning usually about the fourth day. This was followed by erythrocytosis and hemoglobin production. Striking clinical improvement, evidenced by increase in strength and vigor and decrease in diarrhea, coincided with reticulocytosis. No patient showed a marked main hematologic response and each tended to relapse quickly both clinically and hematologically but another injection of vitamin B₁₂ was promptly effective. A single injection of about 100 mcg. probably is necessary to produce the maximal hematologic response in patients as seriously ill as those studied. The patient who received 20 mcg. of vitamin B₁₂ showed spectacular improvement 15 days later following ingestion of 400 Gm. of liver and responded 30 days later to 20 mcg. of pteroyldiglutamic acid per day intramuscularly. The patient given 4 mcg. of vitamin B₁₂ showed little or no hematologic response, but 100 mcg. of pteroyldiglutamic acid per day orally produced a satisfactory response. The 2 patients who received three single injections of 10 to 25 mcg. of vitamin B₁₂ have shown steady clinical and hematologic improvement.

Northwestern University
Chicago, Ill.
School of Tropical Medicine
Pinar del Rio

401. SPIES, T. D., GARCIA LOPEZ, G., MILANES, F., LOPEZ TOCA, R., and CULVER, B.: *The hematologic and clinical response of persons with tropical sprue to vitamin B₁₂*. Rev. internat. de vitaminol. 20: 209-216, 1948.

Reports are presented of 4 cases of tropical sprue in Cuba which were treated with vitamin B₁₂. Three patients received 15 micrograms each [apparently as a single dose], and the fourth 23 micrograms. The number of reticulocytes began to rise on the third to fifth day after treatment and reached a peak of 13.2-26.1 per cent on the seventh or eighth day falling later to 4.4-7.2 per cent. The red blood cell count rose from initial values of 1.41-2.50 million to 2.12-2.70 million on the final day; the corresponding white blood cell counts were 2,900-10,150 rising to 6,350-14,150, and the hemoglobin values, 35-57 rising to 45-60 per cent.

Clinical improvement began a day or two after vitamin B₁₂ was administered. Soreness and redness of the tongue lessened and, after a few days, disappeared. Appetite and strength increased, the number of stools decreased and their character improved. One patient was discharged at his own request on the tenth day the others were observed for twelve days.

402. ADLERSBERG, D.: *Newer advances in sprue*. Oral Surg., Oral Med., & Oral Path. 1: 1109-1118, Dec. 1948.

The treatment of sprue is discussed in a general article. In addition to dietary therapy liver extract or folic acid and vitamin concentrates are given. Crystalline vitamin B₁₂ has a remarkable hemopoietic activity and its role in the treatment of sprue is being studied.

Rock Island Hospital, and
New York, N. Y.

403. SUAREZ, R. M., SPIES, T. D., HERNANDEZ-MORALES, F., and PEREZ, E.: *A note on the effectiveness of vitamin B₁₂ in the treatment of tropical sprue*. Blood 4: 1124-1130, Oct. 1949.

Three patients with tropical sprue were treated over prolonged periods with intramuscular injections of vitamin B₁₂ in order to determine whether full remission might be obtained by giving much larger amounts of the vitamin than are indicated by the dramatic hemopoietic response to minute doses. One patient received a total of 210 micrograms, in nine injections of 10 to 25 micrograms, in a period of 147 days another 205 micrograms in nine injections of 10 to 25 micrograms in 160 days and the third, 200 micrograms in eight 25 microgram injections in 138 days.

None of the patients showed any change for the first three or four days. They began to feel better however on the fourth and fifth day, with the beginning of reticulocyte rise. After the reticulocyte peak had been reached (on the sixth to ninth day) the red blood cells and hemoglobin gradually increased. Each patient slowly gained strength, and the two who had diarrhea showed some improvement in alimentary tract function although the stools did not become entirely normal. Therapeutic responses

have been sustained. It is stated that no agent thus far used in the treatment of tropical sprue has been so effective per unit of weight as vitamin B₁₂.

- 404 RODRIGUEA-MOLINA, R., RAMIREZ-RODRIGUEZ, E. A., ACEVADO C. E., LOPEZ, U., TORRES, J. M., and OLIVELLA, J. A.: *Oral administration of vitamin B₁₂ concentrate in tropical sprue and nutritional macrocytic anemia*. *Acta Haematologica* 6: 277-283, Nov 1951 (abstr. Quart. Rev. Med. 9: 103-104 May 1952)

Authors abstract "Vitamin B₁₂ concentrate, in amounts varying from 25 to 300 µg daily orally administered over periods of 5 to 7 days to two cases of acute sprue and to one case of nutritional macrocytic anemia, produced no significant hematopoietic response, and questionable clinical improvement. One case of chronic sprue presented slight subjective improvement but questionable hematologic response. When 150 and 800 µg. daily were administered to a case of pernicious anemia in relapse, a rapid sustained hematologic and clinical improvement was observed. However no improvement in the long standing neurologic status was noted."

San Juan, Puerto Rico

405. SUAREZ, R. *News and views Foreign newsletter* —*Puerto Rico Blood* 6: 1293-1296, Dec. 1951.

The incidence and treatment of sprue in Puerto Rico are described. A report concerning megaloblastic anemia of pregnancy included one case responding to vitamin B₁₂. Sprue is an ever-present problem. Good results have been obtained with oral folic acid (25 mg. daily) vitamin B₁₂ (15 mcg. weekly by injection) elctrovarum factor and a proprietary mixture of ferrous sulfate, vitamin B₁₂, folic acid, and ascorbic acid. Results were poor or absent when the following were tried ACTH 20 mg. every six hours for 14 days, euphoria but no clinical improvement 500 mg. and 100 mg. oral single doses of vitamin B₁₂ combined folic acid and vitamin B₁₂. A patient with mild symptoms of sprue developed a fulminating degeneration of the posterolateral cord while receiving pteroyldiglutamic acid.

Puerto Rico

406. DIEZ RIVAS, F., HERNANDEZ MORALES F., and MEYER, L. M.: *The oral use of combined vitamin B₁₂ and folic acid in tropical sprue*, *Ann. Int. Med.* 36: 1076-1085, April 1952.

In 6 cases of tropical sprue in relapse the daily administration of 1.67 mg. of folic acid and 25 mcg. of vitamin B₁₂ in tablet form brought about satisfactory hematologic and clinical remission. The results were comparable to those obtained with parenteral liver therapy. When folic acid is given alone the minimal effective oral dose is 10 to 15 mg. a day and that of vitamin B₁₂ is 150 to 200 mcg., but when the two are given simultaneously smaller doses are effective. It appears that the macrocytic anemia of tropical sprue and pernicious anemia is a multiple deficiency state and that both folic acid and vitamin B₁₂ are needed for its correction. The synergistic action of folic acid and vitamin B₁₂ suggests that

folic acid is needed for the proper absorption and utilization of vitamin B₁₂ by the human organism. The extrinsic factor described by Castle may be a combination of substances among which folic acid and vitamin B₁₂ play a prominent role.

University of Puerto Rico School of Medicine
San Juan, Puerto Rico
Mayo Clinic Hospital
Brooklyn, N. Y.

- 407 DIEZ RIVAS, F., SUAREZ, R. M., HERNANDEZ MORALES F., and PEREZ SANTIAGO E.: *The oral administration of vitamin B₁₂ in tropical sprue*. *Ann. Int. Med.* 36: 583-591 Feb. 1952 (Part 2)

The chemistry and source of vitamin B₁₂ are discussed briefly together with parenteral dosage, clinical use of the vitamin and its oral administration in pernicious anemia. Studies in 10 patients with active sprue and definite macrocytic anemias indicate that the oral use of vitamin B₁₂ is not the most efficient or practical way to treat the disease. Small single doses of vitamin B₁₂ (15 to 50 mcg.) by mouth effected a hematopoietic response in 6 of 8 cases (optimal in 1 and suboptimal in 5) and failed in 2. Large single doses (90 to 450 mcg.) failed to induce a response in 3 out of 4 cases. Large multiple daily doses (100 to 400 mcg.) in 2 cases produced a suboptimal response in each case. Definite clinical improvement occurred in 6 weeks in one case and in 12 weeks in the other.

The minimal oral dose of vitamin B₁₂ which will produce an appreciable reticulocytosis and definite clinical improvement when given orally appears to lie between 150 and 200 mcg. daily for two to three weeks or longer. Folic acid, 15 mg. orally daily or 15 mcg. of vitamin B₁₂ intramuscularly is more effective than vitamin B₁₂ orally either with or without gastric juice. Although simultaneous administration of 75 to 150 cc. of gastric juice increased the effect of oral vitamin B₁₂ in 2 of 3 cases, the potentiating effect was less than has been observed in the treatment of pernicious anemia by Castle or at the Mayo Clinic, although larger doses were used. The authors state "This difference may have been due to the difference in the source of the vitamin B₁₂. In this study the Squibb's preparation was used in eight cases, while Castle and Hall and Campbell used vitamin B₁₂ supplied by Merck laboratories." Another explanation offered is that in sprue the intestinal absorption of the extrinsic factors or of the antipernicious anemia factor (vitamin B₁₂) might be impaired.

University of Puerto Rico School of Tropical Medicine
San Juan, Puerto Rico

408. SUAREZ, R. M., SABATER, J., SUAREZ, R. M., JR., and BUSO R.: *The effect of sublingual administration of crystalline vitamin B₁₂ in tropical sprue*, *Blood* 7: 749-754 July 1952.

Authors summary: "Sublingual administration of crystalline vitamin B₁₂ in daily doses of 25 µg. for ten consecutive days failed to induce clinical or hematologic remissions in 5 patients with tropical sprue."

Hospital Mayo
San Juan, Puerto Rico

IDIOPATHIC STEATORRHEA

- 409 TUCK, I. M., and WHITTAKER, N. *Vitamin B₁₂ in idiopathic steatorrhea*, *Lancet* 1 757-759 April 22, 1950

The authors describe 2 cases of megaloblastic anemia associated with idiopathic steatorrhea, one of them was first diagnosed post partum (neither had had any evidence of diarrhea). Neither case responded to adequate intramuscular doses of vitamin B₁₂ which had been proved fully active in Addison's anemia in the dosage given to the present cases (20 to 40 mcg.). Both recovered promptly on therapy with folic acid (20 to 30 mg. by mouth daily for 25 days).

The authors review the work of the other investigators on the use of vitamin B₁₂ in several types of anemia. They state that apparently cases of megaloblastic anemia of pregnancy and refractory cases of megaloblastic anemia with free hydrochloric acid have not been generally investigated for the presence of idiopathic steatorrhea.

These two cases emphasize the importance of looking for a defect in fat absorption in cases of megaloblastic anemia refractory to liver or vitamin B₁₂, even in the absence of characteristic bowel symptoms.

North Middlesex Hospital
England

410. ISRAELS M. C. G., and SHARP J.: *Idiopathic steatorrhea (nontropical sprue) with megaloblastic anemia*, *Lancet* 1 752-757 April 22, 1950

The authors describe in detail 5 cases of idiopathic steatorrhea (nontropical sprue) with megaloblastic anemia, and discuss the diagnostic difficulties—particularly the fact that the fat absorption defect may be missed. Parenteral liver treatment and vitamin B₁₂ in a dose sufficient to produce a full remission in pernicious anemia yielded no response in these cases. All of the patients responded promptly to folic acid, given usually by mouth in a 20- or 10-mg. dose. One patient responded satisfactorily to the synthetic conjugate of folic acid, pteroyl-triglutamic acid, in oral doses of 120 mg. daily. In several of the cases folic acid was given intravenously also, in a dose of 100 or 150 mg. So far blood counts have been maintained by this treatment. In the cases in which they were present, folic acid relieved infantile and amenorrhea without supplementary endocrine treatment. It is concluded that the megaloblastic anemia of nontropical sprue is one of the group of liver-resistant folic acid responsive anemias which includes most of the megaloblastic anemias, with the exception of Addisonian pernicious anemia.

University of Manchester and
Manchester Royal Infirmary
Manchester, England

- 411 COOKE, W. T., PEENEY A. L. F., and HAWKINS, C. F.: *Vitamin B₁₂ in idiopathic steatorrhea*, *Lancet* 1 834, April 29 1950 (in Letters to the Editor)

The writers discuss the fat absorption defect in idiopathic steatorrhea, and state that in their first 100 cases, less than half had symptoms of intestinal disturbance. A

number of the remaining patients had macrocytic anemia which was distinguishable from pernicious anemia only by virtue of a fat absorption defect and the failure to obtain normal blood values with adequate liver extract therapy. In the writers' experience folic acid invariably produces favorable results in megaloblastic anemias associated with idiopathic steatorrhea. Three cases are reported which showed a maximum reticulocyte response after parenteral vitamin B₁₂.

University of Birmingham
Birmingham, Ala.

412. ZUMOFF R. *Possible relationship of folic acid to uric acid metabolism as exemplified by a case of nontropical sprue*, *Am. J. M. Sc.* 225 674-676, June 1953.

A case of nontropical sprue with megaloblastic anemia was found to have hyperuricemia during relapse. The blood uric acid level decreased toward normal after the anemia was successfully treated with folic acid.

The first diagnosis had been pernicious anemia, and both vitamin B₁₂ and liver extract were given, without clinical improvement.

Theoretical and experimental evidence was cited to support the hypothesis that hyperuricemia may sometimes be a chemical sign of folic acid deficiency.

Joseph Hospital of Brooklyn
Brooklyn, N. Y.

OTHER GASTROINTESTINAL CONDITIONS

- 413 SNORF L. D. *Chronic ulcerative colitis*, *M. Clin. North America*: 243-255, Jan. 1951 (Chicago Edition)

Treatment of chronic ulcerative colitis includes papeverine hydrochloride, up to one grain three to six times a day to control intestinal unrest, vitamin B₁₂ parenterally and antibiotics. Antibiotics are indicated where there is evidence of systemic infection: penicillin, in doses of 300,000 to 600,000 units for ten days, has been found to give temporary improvement. Dihydrostreptomycin and aureomycin have been found ineffective, except that aureomycin is of benefit when intestinal involvement extends beyond the bowel wall. Sulfonamides have been generally ineffective, though it is thought they control secondary invasion of the bowel wall by intraluminal bacteria.

Northwestern University Medical School
Chicago, Ill.
Kroner Hospital Association
Evanston, Ill.

- 414 RAIL, G. A. *Two cases of ulcerative colitis treated with vitamin B₁₂*, *Lancet* 2: 816-817 Nov. 8, 1951 (abstr. J.A.M.A. 148 582, Feb. 16, 1952)

Because diets high in liver have been instrumental in healing residual ulcers in cases of anemias, and on the premise that ulcerative colitis might be caused by a dietary deficiency, 2 cases of ulcerative colitis were treated with vitamin B₁₂. Both cases were diagnosed by exclusion, both were presumed to be of about five years standing, and neither patient had ever been treated for ulcerative colitis.

The first patient, a 31-year-old woman, received 20 mcg. of vitamin B₁₂ daily for one week. She was then discharged from the hospital feeling well and having no diarrhea. Injections of B₁₂ were given twice weekly for almost three months. Sigmoidoscopy a month after the start of treatment showed complete healing of the bowel. More than a year later symptoms had not recurred.

In the second patient, a 52-year-old man, vitamin B₁₂ therapy was less intensive. A preliminary five-day course of penicillin plus sulfadiazine cleared up a mass in the left iliac fossa. Thereafter the patient received vitamin B₁₂ plus oral Casydrol (60 minims three times a day). He was discharged from the hospital after four daily injections of 20 mcg. of B₁₂. One month and six injections later the patient had an excellent appetite and no diarrhea. Subsequent treatment was intermittent, and after an additional two and one-half weeks' course of B₁₂ the patient refused further therapy. Some five months after admission sigmoidoscopy showed the bowel to be completely normal. No relapse had occurred to the time of writing, 18 months after the start of treatment.

In summary the author states "Both cases had, at different times before their illness, been on a diet inadequate, in many ways, when compared with that to which they were accustomed."

- 415 CHEWNING C. C., JR. *Colitis following the oral administration of aureomycin and terramycin*, Virginia M. Monthly 75 156-157 March 1952.

The author has recently noted colitis in patients who have been treated with oral aureomycin or terramycin. The colitis was characterized by diarrhea, abdominal pain, edema and erythema of the rectal and sigmoidal mucosae, and in severe cases ulceration of the mucosae. The pathogenesis of this colitis is not understood. *Candida albicans* was not found in the 2 patients whose stools were examined for this organism. The author refers to a personal communication regarding use of vitamin B₁₂ during oral administration of aureomycin; this measure was believed helpful in preventing complications.

Richmond, Va.

- 416 HIRSON C. (*Diabetic nocturnal diarrhea*) Lancet 2 1227 Dec. 29 1951 (in Letters to the Editor)

The writer describes a case of diabetic nocturnal diarrhea of four months duration in which symptoms were controlled by full doses of vitamin B₁₂. Improvement having lasted for two months to the time of writing.

Post House Hospital
Royal Free Hospital, North, England

- 417 QUERLEN and MINOR NOTES *Perianal irritation due to aureomycin*, J.A.M.A. 150 627 Oct. 11, 1952.

A physician requests information on side effects of aureomycin. He has a patient who developed severe perianal irritation after taking 10 capsules of 250 mg. each of aureomycin for a respiratory infection. The editor answers that the irritation in and around the perianal tissues caused by aureomycin and terramycin is dependent on an interference with the normal flora of the colon, the resulting changes in enzyme balance seeming to produce

the irritation. Dr. A. Miltzer of Michael Reese Hospital in Chicago has observed that if these patients are given two teaspoonfuls of an elixer of vitamin B₁₂, folic acid, and ferrous ammonium citrate four times a day with the aureomycin or terramycin, neither diarrhea nor perianal irritation occurs. Dr. Miltzer believes that aureomycin and terramycin destroy the normal flora of the intestinal tract, particularly those organisms which are involved in the synthesis of vitamin B₁₂. It is also pointed out that there is an overgrowth of saprophytes and particularly *Pseudomonas* in patients receiving aureomycin and terramycin, and these organisms contribute to the diarrhea.

- 418 CONWAY N. S., and CONWAY, H. *Vitamin B₁₂ and folic acid in megaloblastic anaemia after total gastrectomy* Brit. M. J. 1 158-161 Jan. 27 1951

The authors report what they believe is the first recorded case of megaloblastic anemia following total gastrectomy to have been treated with vitamin B₁₂. The B₁₂ was injected in the form of the concentrate (prepared from *Streptomyces griseus*) the initial dose being 40 mcg. Striking clinical improvement followed vitamin B₁₂ therapy but the hematologic response was suboptimal compared with that expected in Addisonian pernicious anemia. After 280 mcg. of vitamin B₁₂ concentrate had been given over a period of almost two months, folic acid (20 mg. daily) was given in addition. A complete hematologic remission followed. The authors remark on the fact that the "dumping syndrome" cleared with the improvement in the patient's general condition. They discuss the pathogenesis of the anemia in the light of the classic theory of erythropoiesis. They believe that this type of megaloblastic anemia and Addisonian pernicious anemia are not identical although they are similar morphologically. They discuss the relationship of the anemia described to other megaloblastic anemias with respect to treatment with vitamin B₁₂ and folic acid. Thirty-four references are cited.

University of Glasgow, and
Western University
Glasgow, Scotland

- 419 SWENDSEID M. E., HALSTED J. A., and LIBBY R. L. *Excretion of Cobalt⁶⁰-labeled vitamin B₁₂ after total gastrectomy* Proc. Soc. Exper. Biol. & Med. 83 225-228, June 1953.

Authors summary: "Co⁶⁰-labeled vitamin B₁₂ was administered orally in 0.5 γ doses to 4 patients who had had a total gastrectomy. Each patient excreted in the stools essentially all of the administered radioactivity. When gastric juice was given with the vitamin there was a decrease in Co⁶⁰ excretion to levels similar to those found in control subjects."

Veterans Administration Center, and
University of California Medical Center
Los Angeles, Calif.

- 420 BERMAN J. K., HABEGGER, E. D., and BILLINGS E. *Massive resection of the small intestine a six-year follow-up study* Am. J. Digest. Dis. 20 152-156, June 1953.

After massive resection of the small intestine, a patient suffered from persistent diarrhea, and three and a half years after operation macro-intramuscular administration of

a day brought about a maximum reticulocyte response of 19.7 per cent on the eighth day. Treatment was continued with injection of 15 mcg. twice a week, and the diarrhea responded dramatically. Blood pressure, which had become low after surgery, became elevated again after correction of the anemia with vitamin B₁₂.

*Indonesian General Hospital
Indonesian, Ind.*

- 421 BETHELL, F. H. *Anemias resulting from disturbances of gastrointestinal function*, *Rev Gastroenterol.* 19: 890-896, Nov 1952.

Excerpts from this general article follow: "Anemias resulting from disturbances of gastrointestinal function include the hypochromic microcytic anemias due to iron deficiency and the macrocytic normochromic anemias caused by lack of folic acid or Vitamin B₁₂ and perhaps by other as yet unidentified substances. The daily iron requirement of an adult male is about 1 mg., that of a menstruating female and a rapidly growing child prior to adolescence is approximately 2 mg. The pathogenesis of the macrocytic anemias with megaloblastic marrow reaction has been greatly elucidated within recent years. It is now known that both folic acid and Vitamin B₁₂ are required for normal hemopoiesis and that they participate in wide-spread metabolic processes.

Vitamin B₁₂ is now known to be but one of a group of related compounds for which the generic term cobalamin has been proposed. The Vitamin B₁₂ analogues are present chiefly in sources of animal protein but it is probable that in man appreciable quantities may become available through intestinal synthesis. On the other hand, certain bacteria, including coliform organisms, require for their growth an exogenous source of B₁₂ and so may deprive the host of the vitamin. It has been conclusively demonstrated that the dietary extrinsic factor of Castle is Vitamin B₁₂. The chemical nature of the intrinsic factor has not been elucidated. Evidence has recently been presented which indicates that intrinsic factor may combine with Vitamin B₁₂ to form a microbiologically inactive complex, thereby in some manner facilitating the absorption of the vitamin. Deficiency of Vitamin B₁₂ may result from dietary lack of cobalamin although this appears to be a less common cause of nutritional macrocytic anemia than deficiency of folic acid. Chronic intestinal disorders, including idiopathic steatorrhea, disseminated ileitis, partial obstruction and diminished absorptive surface resulting from short circuiting or resection of small intestine may lead to impaired absorption to both Vitamin B₁₂ and folic acid. Patients who survive for several years after total or subtotal gastrectomy are prone to develop a syndrome indistinguishable from pernicious anemia. Vitamin B₁₂ usually constitutes a complete form of replacement therapy for pernicious anemia, when administered intramuscularly in doses of 10 to 50 micrograms at intervals adjusted to individual needs. Treatment of macrocytic megaloblastic anemias other than pernicious anemia is directed primarily toward correction. If possible, of the underlying disorder. Alleviation of folic acid deficiency usually may be accomplished by the oral administration of 10 mg. daily. The parenteral route should be reserved for patients with intestinal disease. Orally administered Vitamin B₁₂ in daily dosage of 5-10 mcg. may be effective in correcting macrocytic anemia due to dietary lack of this vitamin

when pernicious anemia can be excluded with reasonable certainty."

In the discussion Dr Bethell is asked why folic acid sometimes aggravates neurologic symptoms in pernicious anemia. He replies: "Regarding the effect of folic acid in aggravating the neurologic damage in pernicious anemia, I feel that larger doses of folic acid in the absence of B₁₂ seem to cause true progression of the lesion. On the other hand, we have been impressed by the fact that in the anemias of pregnancy the administration of B₁₂ appears to aggravate the clinical and hematologic manifestations of folic acid deficiency. Animal experiments show that there is a mutual relationship between B₁₂ and folic acid, and in deficiencies of both vitamins, when they are created, the administration of one will be effective for a while but later there will be a rapid aggravation of the deficiency of the one that is not supplied. We do know that Vitamin B₁₂ is apparently essential for the integrity of the nervous system."

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Ann Arbor, Mich.*

422. HALL, B. E.: *Diagnosis and treatment of nutritional anemia*, *J.A.M.A.* 151: 1-8, Jan. 8, 1953.

A review with 45 references on the diagnosis and treatment of nutritional anemias, such as iron deficiency anemias, pernicious anemia, nontropical sprue, megaloblastic anemia of infancy and of pregnancy and the poor peritum, and anemias associated with disease and surgery of the gastrointestinal tract. Nutritional anemias are rather rare in the North Central section of the United States and when seen are usually due to faulty absorption and assimilation of food, rather than to inadequate intake of food. Experiences with and indications for the newer hematopoietic agents, such as vitamin B₁₂ and folic acid, are discussed.

There is no justification for the administration of vitamin B₁₂ or folic acid to patients with iron deficiency anemia: these patients need iron, yet are often treated with vitamin B₁₂ and folic acid, a needless expense.

Parenteral vitamin B₁₂ or liver extracts continue to be the preferred means of treating pernicious anemia. Oral administration of large amounts of vitamin B₁₂ without concomitant administration of gastric juice (which contains intrinsic factor) can elicit a significant hematopoietic response, but the response is usually sub-optimal, as illustrated by a patient who was given 1,000 mcg. of vitamin B₁₂ every second day. A total of 32,000 mcg. of vitamin B₁₂ was given in three months before the erythrocyte count rose to more than 4 million per cu. mm. A similar response could be obtained in eight weeks with 5 mcg. of vitamin B₁₂ by mouth daily plus 150 cc. of human gastric juice. This case illustrates the importance of intrinsic factor in potentiating the absorption and utilization from the alimentary tract of the small amounts of vitamin B₁₂ normally present in foodstuffs. The author emphasizes the fact that folic acid alone should not be used to treat pernicious anemia. Hematologic response is incomplete and the neurologic manifestations of pernicious anemia may develop or progress when the patient receives folic acid only.

In nontropical sprue, absorption of many nutrients is impaired. Therefore, vitamin B₁₂ and folic acid do not

reverse manifestations of the disease that are unrelated to deficiency in hematopoietic substances. Recently other investigations have shown cortisone to be of value in nutritional sprue, apparently by improving the efficiency of intestinal absorption. Cortisone did not improve the hematologic findings; therefore, hematopoietic agents should be given along with cortisone.

Megaloblastic anemia of infancy is due mainly to folic acid deficiency. Its combined treatment with folic acid and ascorbic acid is recommended. The ascorbic acid is apparently necessary for conversion of folic acid into folic acid. It is mentioned that the incidence of megaloblastic anemia of infancy has diminished since

ascorbic acid has been added to proprietary milk products. Megaloblastic anemia of pregnancy and the puerperium does not respond to vitamin B₁₂ but does respond to folic acid.

It is pointed out that megaloblastic anemia rarely follows total gastrectomy. Apparently the stomach is not the only source of intrinsic factor. Megaloblastic anemia sometimes occurs in patients operated on for regional enteritis; 3 such patients are described, one of whom responded to intramuscular vitamin B₁₂ (10 mcg. daily) and the others to folic acid.

*Stanford University School of Medicine
San Francisco, Calif.*

ANIMAL STUDIES

423. BORCH-MADSEN P., and SOEBORG OHLSEN A.: On the effect of total resection of the jejunum and ileum in thriving swine upon the antianemic factor content of liver. *Acta med. Scandinav.* (suppl. 213) 131-91, 1948.

Copenhagen, Denmark

424. CAMERON D. G., WATSON G. M., and WITTS, L. J.: The experimental production of macrocytic anemia by operations on the intestinal tract. *Blood* 4: 803-815, July 1949.

425. DYKE, W. J. C., and HIND H. G.: Bacterial synthesis of vitamin B₁₂ in the alimentary tract. *Lancet* 1: 486-488, March 18, 1950.

The authors found that the vitamin B₁₂ growth-promoting activity for *L. lactis* Dörner and *L. kechumarii* in meat is not increased by incubation with normal gastric juice.

A table is given for the amounts of vitamin B₁₂ present in the alimentary canal at various levels in man, horse, and sheep; it is present in greatest amount below the ileocecal valve.

Purified extracts made from intestinal contents are clinically active in the treatment of pernicious anemia when given parenterally.

The authors suggest that in man the normal requirements of vitamin B₁₂ are met by bacterial synthesis in the colon. They state that some advantage is undoubtedly

to be obtained from supplementation with foodstuffs rich in vitamin B₁₂.

*Essex Biological Institute
Harlow, Chesham, England*

426. HAWK, E. A., and HUNDLEY J. M.: Effect of certain B vitamin deficiencies on gastric secretion in the rat. *Proc. Soc. Exper. Biol. & Med.* 78: 318-322, Oct. 1951.

The effect of vitamin B deficiencies on gastric secretion was investigated in rats subjected to ligation of the pylorus. Acid secretion was sharply reduced by pyridoxine deficiency but there was marked increase in the volume of secretion when pyridoxine was given about 16 hours before the beginning of the secretory test. These findings indicate that pyridoxine plays a previously unsuspected part in the secretory process. Thiamine deficiency produced a marked depression in acid secretion, while riboflavin and pantothenic acid deficiencies produced only slight depressions. In the acute reversal experiments, supplementation with the missing vitamin caused an insignificant rise in the animals deficient in thiamine but not in those deficient in riboflavin. Neither aureomycin nor vitamin B₁₂ had any effect on gastric secretion when added to the complete basal diet, although each caused a slight increase in weight gain. Pepsin secretion was not greatly decreased in any of the deficiencies studied, with the possible exception of thiamine. Pyridoxine deficiency increased both concentration and total output of pepsin.

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Intrinsic and Related Factors

- 427 BERK, L., CASTLE, W. B., WELCH, A. D., HEINLE, R. W., ANKER, R., and EPSTEIN M.: *Observations on the etiologic relationship of achylia gastricae to pernicious anemia. X. Activity of vitamin B₁₂ as food (extrinsic) factor* New England J. Med. 239 911-913 Dec. 9 1948.

Four patients with untreated pernicious anemia were given 5 mcg. of vitamin B₁₂ dissolved in 125 or 150 cc. of physiologic saline solution, daily by mouth, for 10 days. Immediately thereafter they were given the same amount of vitamin B₁₂ daily with 125 or 150 cc. of previously neutralized normal human gastric juice for a second 10-day period. One patient was given 5 mcg. of vitamin B₁₂ a day intravenously during a third period. The reticulocyte rise was low or absent when vitamin B₁₂ was given alone but was present in each case when gastric juice also was given, and in 3 of the patients the reticulocyte response was accompanied by a significant increase of red cells.

These observations indicate that the hemopoietic activity of orally administered vitamin B₁₂ in pernicious anemia is potentiated by simultaneous administration of normal human gastric juice, but that parenterally administered vitamin B₁₂ is still more effective. It is suggested that the food (extrinsic) factor may be identical with or closely related to the anti-pernicious anemia principle of liver which presumably is identical with vitamin B₁₂, and that the gastric (intrinsic) factor is necessary for the optimal utilization of the relatively small amounts of vitamin B₁₂ or related substances present in various foods. Preliminary findings in an untreated patient with pernicious anemia suggest that the focal elimination of vitamin B₁₂ is so great as to create a deficiency which cannot be abolished by the amount of the vitamin derived from the food or synthesized by intestinal bacteria. Other observations suggest that 70 per cent alcoholic extracts of beef muscle, a classic source of extrinsic factor contain a substance possessing both microbiologic activity as vitamin B₁₂ and hemopoietic activity when intravenously injected in patients with pernicious anemia. It is therefore possible that the function of the intrinsic factor of normal gastric juice is to facilitate absorption by the intestine of vitamin B₁₂ or of chemically related compounds in the food, rather than to react with the extrinsic factor as has hitherto been assumed.

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Harvard Medical School
Boston, Mass.
Western Reserve University and
University Hospitals
Cleveland, Ohio*

428. LANDBOE-CHRISTENSEN E., and PLUM, C. M.: *Localization of the anti-anemic principle in the human stomach. Anti-anemic effect of powdered human fundus*, Acta med. Scandinav. (suppl. 206) 130 309-310, 1948 (in Soc. Proc.)
429. BOCCUZZI, G. and PAOLINO W.: *Regulation of hemopoiesis. Importance of the anti-pernicious (anemic) factor in the mechanism of digestive leukocytosis*, Minerva med. 391 139-143, 1948 (abstr. C. A. 42 6910b, Sept. 20, 1948)

Clinical data show a positive correlation between HCl secretion and increase in leukocytes, and between ingested HCl and similar leukocytes, in addition to the phenomenon of retarded leukocytosis, and the capacity of different foods to produce different degree of leukocytosis, according to their content of extrinsic factor

430. GARDNER, F. H., HARRIS J. W., and CASTLE, W. B.: *Erythropoietic activity of extrinsic factor on parenteral administration in pernicious anemia*, Am. J. Med. 7 421, Sept. 1949 (in Soc. Proc.)

Castle at first believed that interaction between the food (extrinsic) and the gastric (intrinsic) factors was necessary for the formation of the anti-pernicious anemia principles of liver—here considered to be vitamin B₁₂. However this reaction does not occur in vitro. The erythropoietic effect of orally administered vitamin B₁₂ has been shown to be enhanced by the simultaneous administration of the intrinsic factor although the same amount of vitamin B₁₂ given alone parenterally is still more effective.

In the present observations a beef muscle extract was used when administered daily by mouth to 8 patients with untreated pernicious anemia. When administered orally every day with 150 cc. of normal human gastric juice it was moderately active, and when administered intravenously without gastric juice it was still more active. Microbiologic assays and erythropoietic effects indicate the presence of somewhat less than 1 microgram of vitamin B₁₂ in each daily dose of the beef muscle extract.

These observations suggest that the extrinsic factor is identical with or closely related to vitamin B₁₂, and that the intrinsic factor is essential merely to facilitate absorption of low concentrations of vitamin B₁₂ present in certain foods other than, for example, liver

- 431 MORGAN E. H., HALL, B. E., and CAMPBELL, D. C.: *Hematopoietic activity of parenterally administered beef muscle concentrate in cases of pernicious anemia*, Proc. Staff Meet., Mayo Clin. 24 594-597 Nov 23, 1949

Since other investigators have shown that beef muscle functions as extrinsic factor the question arose whether its activity might be due to the presence of vitamin B₁₂. Three patients with pernicious anemia in relapse were treated with 20 mg. of beef muscle concentrate a day intramuscularly the amount being derived from 125 Gm. of beef muscle and having approximately 1 mcg. of vitamin B₁₂ activity as determined by microbiologic assay. The hematopoietic responses of 2 of these patients appeared to be optimal, and the response of the third was suboptimal. Since the hematopoietic activity of beef muscle concentrate parenterally administered appears to be similar to that of liver extracts, it is presumably due to the presence of vitamin B₁₂. It seems highly probable that the extrinsic factor in beef muscle is identical with vitamin B₁₂.

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432. CAMPBELL, D. C., HALL, B. E., and MORGAN E. H.: *Oral administration of vitamin B₁₂ in pernicious anemia. II. Studies on the nature and source of intrinsic factor*. Proc. Central Soc. Clin. Research 22: 18, Nov 1949. J. Lab. & Clin. Med. 34 1590 Nov 1949 (in Soc. Proc.)

Since vitamin B₁₂ orally administered depends for its effect on the presence of intrinsic factor (which is believed to be absent in patients with pernicious anemia) the authors made an intensive study of the intrinsic factor in 17 patients with pernicious anemia in relapse. By keeping the quantity of vitamin B₁₂ constant, they were able to test various unknowns for the presence or absence of intrinsic factor. The following observations were recorded:

(1) Intrinsic factor is present in fresh, Berkefeld filtered, pooled human gastric juice and (2) also in extracts of hog stomach and duodenum. (3) It is destroyed by heating at 63 C. for half an hour. (4) It is not lost in human gastric juice with a pH of 1.7 when stored for three months at a temperature of 5 C. (5) Trichloroacetic acid and cold alcohol (95%) precipitates from human gastric juice did not contain the intrinsic factor. (6) In vitro mixtures of pooled human gastric juice and vitamin B₁₂ that had stood at room temperatures and then been heated to 70 C. for half an hour produced no hemopoietic response when given orally to a patient with pernicious anemia. (7) Although the minimal amount of human gastric juice required to produce optimal hemopoietic responses when administered daily by mouth with 5 or 10 mcg. of vitamin B₁₂ was not determined in these experiments, it was found that 75 cc. produced the best results. (8) When large quantities of vitamin B₁₂ alone were administered by mouth, the response varied widely; one patient showed no hemopoietic effect after a single dose of 1,000 mcg. of vitamin B₁₂ concentrate; another showed a suboptimal effect after a single dose of 75 mcg. of crystalline vitamin B₁₂.

These experiments and similar findings by other investigators suggest that the lack of intrinsic factor in patients with pernicious anemia may not be absolute.

Endocrine Unit.

433. GARDNER, F. H., HARRIS, J. W., SCHILLING R. F., and CASTLE, W. B.: *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XI Hematopoietic activity in pernicious anemia of a beef muscle extract containing food (extrinsic) factor upon intravenous injection without contact with gastric (intrinsic) factor*. J. Lab. & Clin. Med. 34 1502-1511, Nov 1949

Seven patients with Addisonian pernicious anemia were given 10 cc. of a 70 per cent alcohol extract of 400 Gm. of beef muscle (as a source of extrinsic factor) by mouth, daily for 10 days or longer. Only one of them showed a detectable reticulocyte response. When four of the patients received the extract together with 150 cc. of normal human gastric juice, all of them showed reticulocyte response. Subsequently the extract was given to 3 of the patients intravenously without additional gastric juice; a further reticulocyte response was observed. The hematopoietic effect of the daily intravenous injection of 10 cc. of beef muscle extract was shown to be less than

that of the daily intramuscular injection of 1 mcg. of crystalline vitamin B₁₂. Ten cc. of the beef muscle extract was shown by microbial assays to contain 0.37 to 0.9 mcg. of vitamin B₁₂ activity. The authors state that the hematopoietic activity of the beef muscle extract when given orally with gastric juice is surprisingly great, judging from observations of the potentiation by normal human gastric juice of 5 mcg. of crystalline vitamin B₁₂. It is therefore suggested that there are substances in beef extract other than vitamin B₁₂ which are potentiated by normal human gastric juice upon oral administration in pernicious anemia.

The gastric (intrinsic) factor was not observed to increase the hematopoietic action of pteroylglutamic acid, nor did it enhance the intestinal absorption of glucose, l-tyrosine, or the digestion products of casein.

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434. HALL, B. E., BETHELL, F. H., MORGAN, E. H., CAMPBELL, D. C., SWENDSEID M. E., MILLER, S., and CINTROV RIVERA, A. A.: *Observation on the presence of intrinsic factor in extracts of hog stomach and duodenum*, Proc. Staff Meet., Mayo Clin. 25 105-113, March 1 1950

Twelve patients with pernicious anemia in relapse were treated with vitamin B₁₂ given by mouth and variously prepared fractions of stomach and duodenum of swine as a source of intrinsic factor. The daily dosage of vitamin B₁₂ was 5 or 10 mcg. and that of the intrinsic factor varied from 0.92 to 8.0 Gm. Extracts of hog duodenal mucosa appear to have greater potentiating activity on vitamin B₁₂ administered orally than do extracts of the entire duodenum.

A thirteenth patient was treated with an acid extract of ground duodenum and vitamin B₁₂ in the form of a complex supplied in capsules each of which contains 9 mcg. of vitamin B₁₂ and the principle present in 0.83 Gm. of acid extract of ground duodenum. The hematopoietic response was fairly satisfactory although probably suboptimal, and clinical improvement occurred but was not as dramatic as that in patients receiving vitamin B₁₂ parenterally. This complex possesses greater heat stability than does the intrinsic factor alone.

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Rochester, Minn.
Shapiro Memorial Institute
Ann Arbor, Mich.
Wayne County General Hospital
Elkton, Md.*

435. LEADING ARTICLE: *Pernicious anemia and the intrinsic factor*. Brit. M. J. 2 612-613 Sept. 9 1950

Pernicious anemia is discussed with relation to Castle's intrinsic factor. The presence of this factor is necessary for absorption of orally administered vitamin B₁₂ from the gastrointestinal tract. The nature of the reaction between the intrinsic factor and vitamin B₁₂, and its specificity and function are as yet not understood. It is believed that the intrinsic factor's action is enzymic. Evidence is cited which shows that several substances are capable of binding vitamin B₁₂, making it gastrointestinal microorganisms. However only

teraction with intrinsic factor is absorption of vitamin B₁₂ from the intestinal tract increased. It is believed that in pernicious anemia the greatly reduced production of intrinsic factor is a result of gastric atrophy and that the vitamin B₁₂ in food is consequently not modified or absorbed.

435. UNGLEY C. C.: *Absorption of vitamin B₁₂ in pernicious anemia. II Oral administration with normal gastric juice*, Brit. M. J. 2 908-911, Oct. 21 1950.

Author's summary and conclusions "The effect of vitamin B₁₂ given with normal gastric juice was observed in eight patients with pernicious anemia. The total amounts were usually 50 µg. + 500 ml. given as a single dose or in divided doses.

"One patient failed to respond to oral therapy. In one who received 40 µg. with only 150 ml. of gastric juice the response was very poor. In two the oral-dose/parenteral-dose ratio was about 10:1 suggesting that about 10% of the vitamin had been absorbed. In the remaining four the response was about as good as would have been expected if the same dose of vitamin B₁₂ had been injected. Thus small doses of vitamin B₁₂ given with normal gastric juice are sometimes efficiently absorbed.

"In these eight experiments each 100 ml. of gastric juice seemed to be enough for the absorption of 0, less than 1, 1 less than 2, 5 or 10, 10 10 and 10 µg. of vitamin B₁₂. Thus the amount of gastric juice necessary to promote the absorption of a given amount of B₁₂ varied considerably. These variations are perhaps only partly due to differences in the amount of intrinsic factor in the samples of gastric juice. Differences in the recipients may have been equally important."

Royal Victoria Infirmary,
Farnborough, Surrey, England

- 437 UNGLEY C. C., and CHILDS, G. A. *Absorption of vitamin B₁₂ in pernicious anemia. III Failure of fresh milk or concentrated whey to function as Castle's intrinsic factor or to potentiate the action of orally administered vitamin B₁₂*, Brit. M. J. 2 911-915, Oct. 21, 1950.

Five cases of pernicious anemia were treated with orally administered vitamin B₁₂, together with fresh milk (2 patients) or whey concentrates (3 patients). The concentration of vitamin B₁₂ used was 10 mcg. in 500 cc. of milk. The whey concentrate had a total volume of approximately 650 cc. which contained 50 mcg. of vitamin B₁₂. Effects of these combinations were compared with those of one or more of the following: parenterally given vitamin B₁₂, normal gastric juice plus B₁₂, B₁₂ plus folic acid, and folic acid plus milk. The results on the blood picture were such that the authors made the following conclusions: "Neither fresh milk nor a whey concentrate in the doses given was an adequate substitute for normal human gastric juice as a source of Castle's intrinsic factor."

Royal Victoria Infirmary,
Farnborough, Surrey, England
Glaxo Laboratories,
Cranford, Middlesex, England

438. UNGLEY C. C. *Absorption of vitamin B₁₂ in pernicious anemia. IV Administration into buccal cavity into washed segment of intestine, or after partial sterilization of bowel*, Brit. M. J. 2 915-919 Oct. 21, 1950.

Studies were undertaken to determine whether vitamin B₁₂ would be effectively absorbed if the possible destructive action of intestinal juice could be reduced or avoided.

When 5 mcg. of vitamin B₁₂ was applied daily in the buccal mucosa of a patient with pernicious anemia, there was a negative response. This patient subsequently responded to 5 mcg. of B₁₂ given orally with gastric juice.

In four experiments in 2 patients, the instillation of vitamin B₁₂ with or without gastric juice into a washed segment of intestine isolated between two balloons of a Miller-Abbott tube resulted in negative or trivial responses. Each patient subsequently responded to vitamin B₁₂ given parenterally or orally with gastric juice.

In a fourth patient, vitamin B₁₂ was given orally after preliminary partial sterilization of the intestine with aureomycin, dihydrostreptomycin, and phthalylsulfathiazole. Even 80 mcg. of B₁₂ was ineffective. This patient later responded to the same dose of B₁₂ given orally with gastric juice. In his conclusion, the author states that these negative findings are against, but do not entirely exclude, the possibility that Castle's intrinsic factor acts by protecting vitamin B₁₂ from destruction in the gastrointestinal tract.

Royal Victoria Infirmary,
Farnborough, Surrey, England

- 439 SCHILLING R. F., FRUTON J. S., HOFSTEE, R. H. J., WELCH, A. D., HARRIS, J. W., GARDNER, F. H., and CASTLE, W. B.: *Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XII Failure of thymus aminopolypeptidase to act as intrinsic factor* J. Lab. & Clin. Med. 36 942-949 Dec. 1950.

In 3 patients with pernicious anemia, daily oral doses of 5 mcg. of vitamin B₁₂ were not potentiated by active preparations of aminopolypeptidase derived from calf thymus, but were potentiated by neutralized normal human gastric juice in amounts of either 10 or 150 cc. daily. This evidence indicates that aminopolypeptidase from calf thymus does not possess intrinsic factor activity as does neutralized normal human gastric juice. The small amount of normal human gastric juice needed to potentiate vitamin B₁₂ suggests that in persons without pernicious anemia the usual clinical methods for determining the volume of gastric secretion would not be adequate to establish or to deny the physiologic adequacy of intrinsic factor.

440. SUÁREZ, R. M., PÉREZ, E., and MAZARIO R. *The failure of urogastrone as a source of the intrinsic factor* Bol. Asoc. méd. de Puerto Rico 43 613, 1951 (abstr. Blood 7 759 July 1952).

"In the hope that some of the gastric juice intrinsic factor may leave the body in the urine of normal persons, the authors tried the simultaneous oral administration of vitamin B₁₂ in 30 µg. daily doses and urogastrone in

doses of 0.15 Gm. daily on a typical case of pernicious anemia showing signs and symptoms of combined system disease. The dialyzed polysaccharide urogastrone failed as a potentiating factor for orally administered vitamin B₁₂.

"Subsequently the case responded well both hematologically and neurologically to the oral use of folic acid in 5 mg. doses three times a day plus the parenteral administration of vitamin B₁₂ in 30 µg. daily doses. The case proves that folic acid can be administered safely to pernicious anemia patients showing nervous system involvement if vitamin B₁₂ is given parenterally at the same time and that urogastrone does not contain Castle's intrinsic factor."

- 441 SCHILLING R. F., HARRIS, J. W., and CASTLE, W. B. *Observations on the etiologic relationship of achylia gastrica in pernicious anemia. XIII. Hematopoietic activity of vitamin B₁₂ (vitamin B_{12a})* Blood 6: 223-232, March 1951

The authors have studied the hematopoietic response to vitamin B₁₂ administered both intramuscularly and orally in 9 patients with Addisonian pernicious anemia. In 2 patients comparisons were made with intramuscular vitamin B₁₂, and in 2 more cases, comparisons were made with oral vitamin B₁₂. The intramuscular dosage of vitamin B₁₂ ranged from 1 to 4 mcg. a day the oral dosage was 5 mcg. a day given first with 50 cc. of water and then with 50 cc. of normal human gastric juice. It was found that vitamin B₁₂ is as potent a hematopoietic agent as vitamin B₁₂ when administered parenterally to patients with pernicious anemia in relapse. It was further found that the hematopoietic activity of vitamin B₁₂ like that of vitamin B_{12a}, and of vitamin B_{12b}, is potentiated by simultaneous oral administration with normal human gastric juice. One of the patients had mild subacute combined degeneration of the spinal cord. Vitamin B₁₂ (1 mcg. daily for 16 days) produced improvement.

*Thorndike Memorial Laboratory
Boston City Hospital, and
Harvard Medical School
Boston, Mass.*

- 442 BETHELL, F. H., SWENDSEID M. E., MILLER, S., and CINTRON RIVERA, A. A.: *Cobalamin (vitamin B₁₂) and the intrinsic factor of Castle* Ann. Int. Med. 35: 518-528, Sept. 1951

A number of chemically distinct forms of vitamin B₁₂ have been isolated. The generic name proposed for the entire group is cobalamin. This appears to be the dietary or extrinsic factor of Castle. Individual members of the cobalamin group have been designated as cyanocobalamin (vitamin B₁₂) and hydroxycobalamin (vitamin B_{12a}). All the cobalamin products tested clinically thus far appear to possess the same type and degree of hematopoietic activity.

The efficacy of liver extracts in the treatment of pernicious anemia appears to depend upon their content of cobalamin, and solutions of cobalamin given parenterally are effective substitutes for liver extracts. Orally administered cobalamin, however, is not efficiently utilized by patients with pernicious anemia, as is shown by a case report. An exogenous source of intrinsic factor is required in such cases when desiccated hog duodenal mucosa was

given the patient responded. Another patient responded to a heated trypsin digest of an extract of duodenal mucosa which had been incubated with 5 mcg. of cobalamin.

In severe macrocytic anemia there may be a deficiency of cobalamin which can be completely rectified by the daily oral administration of 5 mcg. crystalline cyanocobalamin. Reports of 2 patients with macrocytic anemia are presented one of these patients did well on oral cyanocobalamin without other medication; the other who represented a complicated picture of dietary deficiency responded better to parenteral than oral administration, and responded better still when acid was given intramuscularly in addition to cobalamin.

The part played by intrinsic factor in potent orally administered cobalamin is not understood. It does not appear to protect the vitamin from destruction in the alimentary tract nor to prevent its utilization by bacteria in the stomach and small intestine. There seems to be a parallelism between the intrinsic factor activity of gastric and duodenal preparations and their capacity to act with cobalamin, thus preventing the vitamin from inhibiting the growth of microorganisms which require

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Ann Arbor, Mich.
Wayne County General Hospital
Detroit, Mich.*

- 443 GLASS G. B. J., BOYD L. J., RUBINS, M. A., and SVIGALS, C. S. *Relationship of duodenal mucoprotein from human gastric juice to Castle's intrinsic anemic factor* Science 101: 108, Feb. 1, 1952.

Nine patients who received vitamin B₁₂ orally intramuscularly and who also received mucoprotein observed in an effort to understand better the nature of Castle's intrinsic factor. (Apparently vitamin B₁₂ extrinsic factor.) Measurements were made of the red blood cells, hemoglobin, and hemoglobin of the blood and megaloblasts in bone marrow. The authors review the pertinent literature (36 references) and to the following conclusion: "It cannot be stated whether glandular mucoprotein is the pure intrinsic factor or whether the intrinsic factor (enzyme) contained in or adsorbed to its molecule. However the present stage of information, taking into account normal occurrence of glandular mucoprotein in the human gastric juice, the rather homogeneous appearance of this substance on electrophoresis, the extent to which it fits into physiological and pathological data available on the intrinsic factor, the close similarity of physical characteristics of mucoprotein and intrinsic factor, the apparent intrinsic factor activity of mucoprotein, it appears permissible to consider glandular mucoprotein from the gastric dissolved mucin as the main carrier of intrinsic factor activity of the human gastric juice."

*New York Medical College,
Flower and Fifth Avenue Hospitals, and
Westchester Hospital Research Unit
New York, N. Y.*

- 444 GLASS G. B. J., BOYD L. J., and SVIGALS, C. S.: *Potentialization of hematopoietic effect of administered vitamin B₁₂ by glandular mucoprotein of the human stomach*, Federation Proc. 11: March 1952.

"The hematopoietic response of patients with macrocytic anemia to small doses of vitamin B₁₂ administered orally is known to be poor or inconsistent, although the same patients usually respond well to parenteral administration. It has been postulated that this discrepancy was due to the absence of Castle's intrinsic factor from the stomach of patients with pernicious anemia and the consequently deficient absorption of B₁₂ from the gastrointestinal tract. In earlier studies the authors showed that one of the components of gastric dissolved mucus, glandular mucoprotein from fundal glands of human stomach closely resembled intrinsic factor in physical and physiological properties. Moreover this substance was found consistently absent or present only in traces in gastric juices of patients with pernicious anemia. In 7 patients with this disease oral doses of 10-30 mcg. B₁₂ daily were uniformly ineffective. When 50-200 mcg. of glandular mucoprotein daily was added to B₁₂, however, a full hematopoietic response occurred. Recently 2 patients with nutritional macrocytic anemia whose gastric juices contained a normal concentration of mucoprotein were studied. In these patients daily administration of 10-30 mcg. of B₁₂ alone by mouth was followed by a prompt hematopoietic response to normal. These findings suggest the possibility that the presence of glandular mucoprotein in gastric juices may be required for small doses of orally administered vitamin B₁₂ to be effective. Moreover the quantitation of glandular mucoprotein in gastric juice may be of value in the differentiation of various types of macrocytic anemia capable of responding to B₁₂."

New York Medical College, and
Princess and Peter A. B. Award of Excellence
New York, N. Y.

445. TOWNSEND S. R. *Pernicious anaemia due to deficiency of extrinsic factor* Canad. M. A. J. 67: 53-54, July 1952.

A case of pernicious anemia believed to be the result of deficiency in protein which caused deprivation of the extrinsic factor was successfully treated with vitamin B₁₂ by mouth.

McGill General Hospital
Montreal, Canada

446. MERSHEIMER, W. L., GLASS, G. B., SPEER, F. D., WINFIELD, J. M., and BOYD L. J.: *Gastric mucin—a chemical and histologic study following bilateral vagotomy gastric resection and the combined procedure*, Ann. Surg. 136: 668-679 Oct. 1952.

The effect of surgical procedures (partial gastrectomy bilateral vagotomy or both) on the pattern of hydrochloric acid and glandular mucoprotein secretion following insulin or histamine stimulation was studied.

In the discussion on this article, Dr. Glass points out that disturbances in the secretion of glandular mucoprotein following vagotomy and gastrectomy may be of significance in interpreting the hematologic disturbances which may or may not occur after operations on the stomach. Dr. Glass' studies indicate that glandular mucoprotein is closely related to Castle's intrinsic hematopoietic principle. It is present in all normal gastric juices but is absent or present only in traces in the stomach of patients with pernicious anemia. Normal individuals re-

spond to administration of insulin with a rise in mucoprotein and acid concentration, but in patients with pernicious anemia the response of the acid as well as that of glandular mucoprotein to vagal stimulation is abolished. In studies on 9 patients with pernicious anemia in relapse it has been shown that glandular mucoprotein probably is needed for the utilization of orally administered vitamin B₁₂. In the absence of mucoprotein the oral ingestion of vitamin B₁₂ is practically without hematopoietic effect. However when glandular mucoprotein processed from normal human gastric juice is added to small oral doses of B₁₂, a hematopoietic effect is obtained. Glandular mucoprotein is produced by the fundic glands of the stomach. Thus in patients with partial gastrectomies in which the fundus is preserved, an ensuing macrocytic anemia need not be expected since mucoprotein would still be secreted. After total gastrectomy on the other hand, no mucoprotein is available. This is probably why macrocytic anemias have been noted with increasing frequency after total gastrectomy in patients not treated with liver or vitamin B₁₂ injections, provided that they do not eat liver and live for more than three years. After this period the stores of the hematopoietic principle become exhausted and macrocytic anemia may develop.

New York Medical College
New York, N. Y.

447. MARMION, B. P., GARDNER, H. J., SAINT E. C., and STUBBE, J. L. *Gastric mucoprotein and intrinsic factor response of pernicious anemia to oral vitamin B₁₂ plus normal gastric juice treated with receptor-destroying enzyme*, Lancet 1: 273-274, Feb. 7 1953.

Authors summary "A young woman with classical pernicious anemia in relapse was treated with oral vitamin B₁₂ [10 mcg. per day] and human gastric juice acted upon by receptor-destroying enzyme (R.D.E.). A normal therapeutic response was obtained.

"Although there is good evidence that intrinsic factor in gastric juice is a mucoprotein, it is not one which is inactivated by R.D.E., thereby differing in its properties from gonadotrophin and mucoproteins derived from respiratory-tract cells."

Wilkes and Elms Path Institute of Medical Research, and
Royal Edinburgh Hospital
Edinburgh, Scotland

448. GLASS, G. B. J. *Hematopoietic activity of glandular mucoprotein from human gastric juice*, Gastroenterology 23: 219-233, Feb. 1953.

Authors conclusions "Glandular mucoprotein, secretory product of mucous cells of the stomach, acts as a potentiator of the hematopoietic activity of vitamin B₁₂ on its oral intake. It is yet undetermined by which mechanism it facilitates the intestinal absorption of vitamin B₁₂ and whether glandular mucoprotein is identical with Castle's intrinsic hematopoietic factor or whether it acts as its carrier in which Castle's factor is bound.

"The participation of glandular mucoprotein in the metabolism of vitamin B₁₂ does not preclude the existence of other factors influencing the effects of vitamin B₁₂ upon hematopoiesis, such as folio-folic acid axis or the role of the specific inhibitor of erythrocytes maturation in pernicious anemia.

"With the absence of glandular mucoprotein from the stomach, which is the case in pernicious anemia, the hematopoietic effect of vitamin B₁₂ administered by mouth in small or moderate doses is very weak or none. The addition of glandular mucoprotein to by itself inefficient small oral doses of B₁₂ results in a striking hematopoietic response. With the presence of glandular mucoprotein in the gastric juice in patients with macrocytic non-pernicious anemia the oral administration of small doses of B₁₂ alone may cause a hematopoietic response, provided that the cases are suitable for the treatment with B₁₂."

"The conclusive evidence for the significance of the presence of glandular mucoprotein in the stomach for the ability of patients with macrocytic anemia to respond to the oral treatment with B₁₂ is yet to be obtained. It appears, however, that the presence or absence of glandular mucoprotein in the gastric juice may be helpful in differentiation of various forms of macrocytic anemias."

New York Medical College and
Flower and Clark Medical Research
New York, N. Y.

- 449 WALLERSTEIN, R. O., HARRIS J. W., SCHILLING, R. F., and CASTLE, W. B. Observations on the etiologic relationship of achylia gastrica to pernicious anemia. XV Hematopoietic effects of simultaneous intravenous and of simultaneous or serial oral administration of intrinsic factor and vitamin B₁₂. *J. Lab. & Clin. Med.* 41 363-375, March 1953.

Authors summary and conclusions: "Observations were made upon fifteen patients with pernicious anemia under controlled dietary conditions. No consistent evidence was obtained for potentiation of the hematopoietic effect of vitamin B₁₂ by simultaneous daily intravenous injection with intrinsic factor. A reciprocal rather than a stoichiometric relation exists between vitamin B₁₂ and intrinsic factor with respect to the potentiation of vitamin B₁₂ by intrinsic factor upon simultaneous daily oral administration."

"The hematopoietic effect of the daily oral administration of 1 µg. of vitamin B₁₂ may be detectably increased by as little as 10 ml. of normal human gastric juice. However the hematopoietic effect of 1 µg. of vita-

min B₁₂ and 50 ml. of gastric juice does not equal that of the daily injection of 1 µg. of vitamin B₁₂ alone. When separated by an interval of three or four hours the hematopoietic effect of the serial administration of vitamin B₁₂ and of intrinsic factor is greater when the intrinsic factor precedes the vitamin B₁₂. These and other recent clinical observations suggest that the essential physiologic function of intrinsic factor is merely to increase somewhat the assimilation of vitamin B₁₂. They indicate the possibility that the primary effect of intrinsic factor is upon the intestinal wall rather than upon the vitamin B₁₂."

Boston City Hospital, and
Harvard Medical School
Boston, Mass.

450. PRATT P. T., and JOHNSON M. O.: Pathogenesis and treatment of pernicious anemia, *Nebraska M. J.* 38 84, 87 March 1953.

A review of the literature on the pathogenesis of pernicious anemia shows the defect to be an inability to absorb vitamin B₁₂ from the gastrointestinal tract. Maturation of the nucleus of the red blood cell involves the changing of uracils into thymine, which is potentiated by the folate acid-folate acid mechanism, and the change from thymine to thymidine, which is potentiated by vitamin B₁₂. The formation of hemoglobin is dependent on iron metabolism. Vitamin B₁₂ deficiency slows nuclear growth—larger but fewer red cells are formed; there are also fewer leukocytes and thrombocytes, and nerve cell metabolism may be affected. The life span of the red cell is apparently reduced when vitamin B₁₂ is inadequate.

The authors have seen 11 patients with pernicious anemia treated with vitamin B₁₂ at the University Hospital and Hematology Clinic since January 1, 1950. In patients in relapse, clinical improvement has been maintained by the equivalent of 1 or 2 meg. of vitamin B₁₂ a day administered as a total dose every two to three weeks. In neurologic pernicious anemia the dose is increased in amount and frequency until no further improvement is obtained or until evidence of neurologic disease is gone, usually for more than a year after which the patient is placed on a maintenance dose.

University of Nebraska College of Medicine
Omaha, Neb.

ANALYTICAL STUDIES

451. TERNBERG J. L., and EAKIN R. E.: Erythrin and apoerythrin and their relation to the antipernicious anemia principle. *J. Am. Chem. Soc.* 71 3858, Nov 1949

Normal gastric juice contains a nondialyzable, heat labile substance (apoerythrin) which combines with erythrin (vitamin B₁₂) to form a nondialyzable complex (erythrin). In this combination erythrin is not available to microorganisms, but it is released by heat. Hog gastric mucosa preparations contain a principle which appears to be analogous to apoerythrin. The principle has been isolated and studied. It is probable that apoerythrin is the intrinsic factor of Castle or a component thereof.

University of Texas and
Cotton Foundation for Research
Austin, Texas

452. WOLF D. E., WOOD T. R., VALIANT J., and FOLKERS K.: Vitamin B₁₂ and the intrinsic factor. *Proc. Soc. Exper. Biol. & Med.* 73 15-17 Jan. 1950.

Studies of possible chemical reaction between crystalline vitamin B₁₂ and gastric juice showed no evidence for such a reaction. No evidence of gross structural change in vitamin B₁₂ by reaction between this vitamin and the gastric juice intrinsic factor was found. A possible reaction resulting in a very small structural change of the large vitamin B₁₂ molecule, which could produce as little as 5 meg. of orally active product, is being investigated. A concentrate prepared from beef muscle having vitamin B₁₂-like microbiologic activity was found to have hematopoietic activity when administered parenterally to pernicious anemia patients; thus, the

factor acts like vitamin B₁₂. These results support the previous hypothesis that the effect of the intrinsic factor is due to its ability to promote the absorption of the small amounts of vitamin B₁₂ or its equivalent which occur in natural foods.

Worsh & Co., Inc.
Rahway, New Jersey

453. MEYER, C. E., EPPSTEIN, S. H., BETHELL, F. H., and HALL, B. E. *Nature of the intrinsic factor* Federation Proc. 9 205, March 1950.

"During the course of concentrating the intrinsic factor from hog intestines, certain similarities between this factor and lysozyme became apparent. It has been observed that lysozyme derived from egg white has the property of combining with vitamin B₁₂, thus rendering it unavailable to *Lactobacillus lactis* Dornier and *Escherichia coli*. Oral administration of 9 µg. of vitamin B₁₂ in the form of the complex, daily to patients with pernicious anemia results in remission, with characteristic reticulocyte response and erythropoiesis."

The Upjohn Company
Kalamazoo, Mich.
Research Research Institute
Ann Arbor, Mich.
New York
Executive Office

454. RIDING, D.: *Vitamin B₁₂*, Brit. M. J. 2 348, Aug. 5, 1950 (In Soc. Proc.)

It is suggested that in man the normal requirements of vitamin B₁₂ are met by bacterial synthesis in the colon, and that many of the fecal organisms may be capable of synthesizing this vitamin.

The author states that normal gastric juice contains a nondialysable, heat-labile substance which combines with vitamin B₁₂ to form a complex which is nondialysable and not dissociated by dialysis. The B₁₂ in this complex is unavailable to microorganisms, but, upon being released by heat, is again microbiologically active. This inhibitor is believed to be Castle's intrinsic factor or a fraction thereof. The intrinsic factor has been found in large quantities in all specimens of human gastric juices containing free hydrochloric acid. Pernicious anemia patients do not have it. Of the animals, the dog and the pig possess the intrinsic factor; the horse, calf and sheep do not.

Since orally given vitamin B₁₂ produces a response in cases of pernicious anemia, it is possible for man to use this vitamin without the presence of this intrinsic factor. It is conjectured that the intrinsic factor may act as a protective mechanism. It is suggested that the complex formed by B₁₂ and the intrinsic factor might also prevent destruction of this vitamin by the alkaline digestive juices of the upper intestine.

Kenner, England

455. HALL, B. E., MORGAN, E. H., and CAMPBELL, D. C.: *Studies on the nature of the intrinsic factor of Castle*, Brit. M. J. 2 585-589 Sept. 9 1950.

In the therapy of pernicious anemia, orally administered vitamin B₁₂ requires for its effective utilization the presence of a potentiating agent (intrinsic factor of

Castle). In the absence of this factor hemopoietic responses are variable and unpredictable when the vitamin is given orally in doses up to 100 times the minimal effective parenteral dose. Known sources of the intrinsic factor include gastric juice of normal or elevated acidity from human beings, and extracts of the stomach and intestines of swine. It has not been possible to prepare concentrated preparations which yield consistently effective and reliable therapeutic results because of ready destructibility during processing. The authors believe that their studies support the concept of the enzymic nature of the intrinsic factor.

Three hypotheses are presented to explain the role of the intrinsic factor which is probably concerned with the absorption of vitamin B₁₂. 1) It may alter the chemical or physical nature of vitamin B₁₂ so that it traverses the intestinal barrier more readily. 2) It may protect the vitamin from destruction by secretions of the digestive tract, and 3) It may prevent the removal of the vitamin from the contents of the upper part of the gastrointestinal tract by microorganisms. Certain observations are cited with respect to the third hypothesis. 1) In cases of pernicious anemia a great increase has been found in gastrointestinal tract concentrations of *Escherichia coli* and *Clostridium welchii*. 2) Normally certain levels of the small intestine are relatively free of bacteria. 3) Recently Davis and Mingioli (personal communication) have shown that *E. coli* has an avidity for vitamin B₁₂. 4) Lichtman, Ginsberg, and Watson (unpublished data) have reported that the daily oral administration of 3 Gm. of aureomycin and 3 mcg. of vitamin B₁₂ to patients with pernicious anemia in relapse resulted in a hemopoietic response. Since aureomycin would be expected to reduce the number of colonic organisms in the gastrointestinal tract, it is believed that vitamin B₁₂ may be made more available for absorption.

New York
Rockefeller Museum

456. NOER, B. *Bacterial synthesis of vitamin B₁₂*, Lancet 2: 706, Dec. 2, 1950 (In Letters to the Editor)

The writer reports bacteriologic investigations of the use of mucosa of fresh pig stomach and of three commercial preparations of pig stomach against pernicious anemia. A constant finding has been the presence of only two types of bacteria in considerable numbers—*Bacillus subtilis* and one of the nonpathogenic corynebacteria. The writer presumes that one of these bacteria may be identical with the "intrinsic factor." Both bacteria have been found to synthesize vitamin B₁₂ in significant amounts. It is stated that, although clinical investigations with strains of these bacteria in cases of pernicious anemia have not given convincing results, these bacteria are able to synthesize not only vitamin B₁₂, but also antibiotics which are instrumental in the control of undesirable bacterial growth in the intestinal mucosa in these cases.

The following working hypothesis of the etiology of pernicious anemia is proposed: "If vitamin B₁₂ is to be absorbed it must be present in the intestinal mucosa in a concentration higher than in the tissues. This is the case in the normal organism, where the intestinal mucosa is infected with B₁₂-producing strains. In the diseased organism these strains are expelled from the mucosa and replaced by B₁₂-consuming *Bact. coli* and *Lactobacilli*,

and this mucosa forms a barrier to the absorption of B₁₂ from the intestinal contents."

Further investigations are under way

Forsman, Ltd.
Copenhagen, Denmark

457. BEERSTECHEER, E., Jr. *A biologically active product of vitamin B₁₂*. Federation Proc. 10 161, March 1951.

"Thermal treatment of crude sources of intrinsic factor produces an intrinsic factor-uncombinable thermolabile factor which stimulates the growth of bacteria in the presence of vitamin B₁₂ under assay conditions which have hitherto been found to produce a response only to vitamin B₁₂. This response is considerably greater than that produced by similar amounts of the vitamin. The data suggest that this factor is a normal product of vitamin B₁₂ and is probably the erythrocyte maturation factor (EMF). Since the factor is inactive in the absence of the vitamin, it appears that vitamin B₁₂ has more than a single metabolic product critical for growth. The evidence indicates that intrinsic factor combines with vitamin B₁₂ to produce a complex, the erythrocyte of Eakin and Ternberg (*J. Am. Chem. Soc.* 71 8858, 1949 [Abstr. 451]) which is normally enzymatically degraded to form the active principle, thus explaining the inability of vitamin B₁₂ synthesized in the gut to manifest a hematopoietic response. The chemical properties of the active principle are being characterized."

University of Texas
Houston, Texas

458. BIRD O. D., and HOEYET B. *The vitamin B₁₂-binding power of proteins*. *J. Biol. Chem.* 190 181 189 May 1951.

Authors summary "A variety of proteins, including intrinsic factor concentrate and lysozyme, bind vitamin B₁₂ when measured by adding them unheated in increasing amounts to tubes of vitamin B₁₂ assay medium containing known amounts of vitamin B₁₂ and noting the inhibition of growth of *Lactobacillus leichmannii*."

"This inhibition method indicated that intrinsic factor concentrates bind vitamin B₁₂ compounds but not thymidine, whereas lysozyme binds vitamin B₁₂ and thymidine."

"A method of measuring vitamin B₁₂-binding based on the non-dialyzability of the bound vitamin indicates a much higher capacity of intrinsic factor concentrate to bind vitamin B₁₂ than is shown by the inhibition method. This dialysis method indicated that lysozyme does not truly bind vitamin B₁₂, whereas intrinsic factor concentrate does, since the added vitamin is dialyzed from the former but not the latter. The method is also more suitable than the inhibition method for determining the vitamin B₁₂-binding power of crude sources of active protein which contain relatively large amounts of non-vitamin B₁₂ growth factors for *L. leichmannii*."

Parke, Davis and Co.
Ann Arbor, Mich.

459. GORDIN, R.: *Studies on Castle's extrinsic factor*. *Acta. med. Scandinav.* 140 199. July 23, 1951 (abstr. J.A.M.A. 147 1890, Dec. 1, 1951)

"To trace and produce in the purest possible form a substance acting as an extrinsic factor experiments were performed on 17 patients with cryptogenic pernicious anemia who were given substances other than ordinary meat by mouth, with gastric juice used as the intrinsic factor. The substances used as the extrinsic factor were peptone, fibrin hydrolysate (aminocoll[®]) crude meat extract, pure meat extract, raw ground liver liver extract ("heptomin") and vitamin B₁₂. A good effect was obtained with peptone, different meat extracts, small quantities of liver and liver extract, and small doses (5 mcg.) of vitamin B₁₂. Tests with fibrin hydrolysate gave less satisfactory results. The amount of intrinsic factor contained in 40 ml. of normal gastric juice proved sufficient as a daily dose. Chemical analyses of fibrin hydrolysate and peptone demonstrated that these substances do not contain cobalt. Consequently the part played by cobalt in the extrinsic factor effect, as suggested by Smith and Rickes, with respect to vitamin B₁₂, seems to be debatable."

460. EAKIN R. *Apoerythrin*, *Chem. & Engin. News* 29 5450 Dec. 24, 1951 (in Soc. Proc.)

At the seventh ACS Southwest Regional Meeting "Apoerythrin was the name applied to a protein factor which binds vitamin B₁₂. The important characteristic of this material is that it may be administered orally in pernicious anemia cases where previously injection of antianemia principles has been required."

461. CALLENDER, S. T., and LAJTHA, L. G. *On the nature of Castle's hematopoietic factor*. *Blood* 6 1234-1239 Dec. 1951

Experiments in vitro support the theory that Castle's hematopoietic factor is a thermolabile combination of normal gastric juice (intrinsic factor) and vitamin B₁₂ (extrinsic factor). In vitro a combination of crystalline B₁₂ and gastric juice as well as normal serum matured megakaryoblasts as normoblasts. Neither gastric juice nor crystalline B₁₂ alone affected megakaryoblasts. It was also found that the hematopoietic in normal serum is thermolabile.

Radcliffe Infirmary
Oxford, England

462. HOFF JØRGENSEN E. *The effect of "intrinsic factor" on the absorption of vitamin B₁₂ by wild type of *Escherichia coli**. *Arch. Biochem. & Biophysics* 36 235-236, 1952 (abstr. *Blood* 7 857 Aug. 1952)

"Although wild type *E. coli* does not require vitamin B₁₂ as a growth factor the organism will absorb a large amount of B₁₂ from the medium. Extracts of pig stomach inhibit this absorption. Lysozyme is without effect. This may offer an explanation of the effect of 'intrinsic factor' as it is known that in patients suffering from pernicious anemia large amounts of *E. coli* are found in the upper intestine and even in the stomach."

University of Copenhagen
Copenhagen, Denmark

463. WELCH, A. D., SCHARF V., HEINLE, R. W., and MEACHAM, G. C.: *Assay for intrinsic factor in patients with pernicious anemia in remission given radioactive vitamin B₁₂*. *Federation* 303-309 March 1952.

"Orally administered vitamin B₁₂ produces little or no hematopoietic effect in patients with pernicious anemia unless intrinsic factor is administered simultaneously. The presence of materials with vitamin B₁₂-activity presumably formed by bacteria in the intestine, has precluded the determination in the feces of unlabeled ingested vitamin. Studies with cyanocobalamin-Co⁵⁷ (Merrick & Co.) the gamma radiation of which can be measured in the presence of feces by scintillation counting, have shown that patients with pernicious anemia in remission given oral doses of only 0.5 µg. (0.03 µc.) of the radioactive vitamin, excrete 70-95% of the cobalt in the feces. When gastric juice or concentrates of hog stomach are administered simultaneously with the vitamin, the amount of cobalt excreted is reduced to 5-30% of that ingested. A typical patient given 3 doses of radioactive vitamin B₁₂ (0.5 µg.) orally without intrinsic factor eliminated 70, 95 and 95% respectively of the cobalt concurrent administration of 10 mg. of a hog stomach fraction reduced the elimination to 25% and a greater effect was not produced by a dose of 100 mg. The findings in all patients suggest that intrinsic factor is involved in the removal of the vitamin from the gastrointestinal contents and form the basis of a technique for assay of intrinsic factor fractions. The methods are simple and relatively rapid as compared with the classical assay procedure requiring prolonged clinical and hematologic observations in seriously ill, untreated patients."

Western Reserve University School of Medicine
Cleveland, Ohio

- 464 BURKHOLDER, P. R.: *Microbiological studies on materials which potentiate oral vitamin B₁₂ therapy in Addisonian anemia*, Arch. Biochem. & Biophysics 39 323-332, Aug 1952 abstr J Clin. Nutrition 1 171, Jan 1953.

Author's summary "Strains of bacteria were isolated from stomach and jejunal juice of Addisonian anemia patients, and most of these bacteria were found to have a strong tendency to absorb vitamin B₁₂ from solution. Concentrates prepared from hog stomach mucosa when added to incubating mixtures prevented the consumption of vitamin B₁₂ by the bacteria. Our studies support the theory that typical Addisonian anemia is a disease caused by removal of vitamin B₁₂ from the patient's digestive tract by a greatly increased microbial flora under conditions of achlorhydria and lack of intrinsic factor in the gastric region.

"Preparations of swine mucosa showing high potency in rendering vitamin B₁₂ unavailable to bacteria are also rich in intrinsic factor activity as determined with adequate doses administered along with vitamin B₁₂ orally to patients, with resultant characteristic hematopoietic responses. Any conclusion concerning the identity of vitamin B₁₂ absorption factor with the intrinsic factor of Castle awaits chemical purification and demonstration of both microbiological and clinical potency in the purified material."

The mode of action of both vitamin B₁₂ and intrinsic factor is described and substantiated by 19 references.

Oakland, Richmond Laboratory
Tide Corporation
New Haven, Conn.

- 465 CHOW B. F., and YAMAMOTO R.: *Properties of B₁₂ binding substance in gastric juice*, Federation Proc. 12 189 March 1953.

"A substance (X) in gastric juice forms with vitamin B₁₂ a complex not easily dissociable by dialysis or absorption with resting microorganisms. This complex could be tagged isotopically with B₁₂ labeled with Co⁵⁷ thus facilitating separation by discarding fractions without radioactivity. Such studies yielded the following results: a) *Physico-chemical characteristics*. The complex was soluble in 40% aqueous alcohol but completely precipitable at 60%. It possessed a minimum solubility at pH 3.5-4.0. Isoelectric precipitation yielded a purified fraction with an approximate molecular weight of 5×10^5 by diffusion measurement. It contained more than one fraction by paper chromatography. b) *Importance of acidic group in B₁₂ binding*. The presence of strong acidic groups in (X) suggests the possibility of other compounds with similar groups to bind with B₁₂. Sulfonated polysaccharides, by dextrorbons or proteins were also capable, to varying degrees, of reducing the uptake of B₁₂ by microorganisms and releasing it from tissues and, in some instances, aided in the absorption. c) *Binding powers of gastric juice from individuals with pernicious anemia and from old subjects*. Radioactive B₁₂ bound to a given amount (N or volume) of gastric juice varied quantitatively according to the source of the latter. Gastric juice from subjects with pernicious anemia (adult or infantile) lacked B₁₂ binding power and that from certain groups of the aged (65 years or older) combined with only a small amount, when compared with healthy adults."

Johns Hopkins University
Baltimore, Md.

- 466 GLASS G. B. J.: *Gastric mucus and its constituents: some physicochemical characteristics cellular origin, and physiological significance*, Gastroenterology 23 636-658, April 1953.

This article is devoted mainly to the chemistry and physiology of gastric mucus. Glandular mucoprotein is a constituent thereof. Brief mention is made of 7 patients with pernicious anemia who showed no or very slight hematologic response to daily administration of 10 to 50 mcg. of oral vitamin B₁₂ daily. When 50 to 200 mg. of glandular mucoprotein was added daily to the same oral dose of vitamin B₁₂, a strong hematopoietic response was obtained.

Rose York Medical College
New York, N. Y.

- 467 SWENDSEID M. E., SHAPIRO H., and HALSTED J. A.: *A missing link may be pernicious anemia factor*, Science News Letter 63 296, May 9 1953.

"A missing link in a nutritional chain involved in the manufacture of red blood cells may be an important factor in pernicious anemia.

"In the research a substance known as the B-12 binding factor which is present in the gastric and duodenal contents of normal subjects, could not be found in the duodenum of persons with pernicious anemia.

"Although the exact role of the binding factor is still unknown, it is thought that it may be essential in the

absorption and utilization of vitamin B-12. Vitamin B-12 plays an important role in the manufacture of red blood cells, and thus the lack of the binding factor could interfere with this process.

"The study also indicated that persons who have had their entire stomachs removed by surgery may not be able to absorb enough B-12 for normal nutrition. The binding factor was not found in duodenal contents of such patients examined in the investigation."

*University of California at Los Angeles Medical School, and
Los Angeles VA Center
Los Angeles, Calif.*

468. PRUSOFF W. R., WELCH, A. D., HEINLE, R. W., and MEACHAM, G. C.: Concentration of intrinsic factor and vitamin B₁₂-binding activities of fractions of deacidified hog stomach. *Blood* 8: 491-501 June 1953.

Authors summary "Fractionation of deacidified hog stomach through the use of isoelectric ammonium sulfate precipitation techniques, and *in vivo* assays in patients with pernicious anemia in relation to daily oral doses of 5 µg. of vitamin B₁₂, has indicated a concentration of intrinsic factor activity in an active fraction equivalent to 0.03 per cent of the original crude material. The electrophoretic patterns of the active fraction indicated a lack of homogeneity."

"The vitamin B₁₂-binding activities of three fractions obtained by precipitation with ammonium sulfate were investigated by microbial inhibition and dialysis. There was no correlation between the ability of fractions to bind vitamin B₁₂ and to exert intrinsic factor activity. Among these fractions, that with the highest intrinsic factor activity in patients with pernicious anemia bound the least amount of vitamin B₁₂."

*Western Reserve University School of Medicine, and
Cleveland Hospital
Cleveland, Ohio*

Nutrition, Growth and Metabolism

VITAMIN DEFICIENCY

- 469 BETHELL, F. H. *Nutritional factors concerned in blood formation*, Chicago M. Soc. Bull. 51 710-711, March 12, 1949

One of the nutritional factors briefly reviewed in this article is vitamin B₁₂. Its relationship to folio acid is not yet clear. The deficiency of these vitamins in the body is followed by macrocytic anemia and granulocytopenia. It may be that the conjugated form of folio acid supplied by food must be converted to the free form by specific enzymes or conjugates before it can be utilized, and that in patients with pernicious anemia there is a relative inability to convert or split the conjugated to the free form. The feces of patients with pernicious anemia contain relatively large amounts of vitamin B₁₂, which may mean that the vitamin is synthesized by bacteria in the bowel where absorption does not occur or that there is a specific absorption defect in the patients. The author concludes "It seems possible that the extrinsic factor of Castle is a dietary complex containing vitamin B₁₂ in a conjugated form or as a precursor substance and that the intrinsic factor is an enzyme concerned with the conversion of the vitamin into a form in which it can be absorbed or utilized."

470. SPIES, T. D.: *The control of pellagra*, Rhode Island M. J. 32: 431-435, Aug. 1949

This paper includes a discussion of nutritional macrocytic anemia. Vitamin B₁₂ is said to be the most effective anti-anemic substance known, per unit of weight. It is effective in pernicious anemia, nutritional macrocytic anemia, and tropical sprue, diseases which are often associated with pellagra.

*Illness Hospital
Birmingham, Ala.*

471. WISSMER, B.: *Les facteurs alimentaires dans l'hématopoièse (Alimentary factors in hematopoiesis)* Presse méd. 57 898-900, Oct. 15, 1949

The author reviews the various dietary elements which take part in the elaboration of hemoglobin and erythrocytes. Iron, copper and folio acid are usually present in sufficient amounts, but in Biermer's anemia (pernicious anemia) the organism cannot transform folio acid from its conjugated form to a utilisable form. Vitamin B₁₂ may be the extrinsic factor. Its action appears to be very powerful. The other vitamins are necessary in general but not individually; this is also true for amino acids. The role of proteins has been demonstrated, particularly in severe berne, anemia of pregnancy and tropical anemia. In conclusion, the author suggests that the proteins supplied in the diet seemingly are reduced to simple substances, stored and resynthesized in special organs such as the liver. It is probably from this source of endogenous proteins that amino acid precursors of the globin molecules and protein stromes are formed.

Geneva, Switzerland

472. MORRIS, J. E., HARPUR, E. R., and GOLD-BLOOM, A.: *The metabolism of tyrosine in infantile scurvy* J. Clin. Investigation 29 325-335, March 1950.

Increased urinary excretion of tyrosine and its deaminated derivatives, tyrosyl and hydroxyphenyl, was demonstrated in 6 male infants with scurvy or preclinical scurvy following the ingestion of tyrosine (1 Gm./Kg. body weight/day incorporated in a formula providing approximately 3.5 Gm. of cow's milk protein/Kg. daily). The urinary excretion of hydroxyphenyl varied. Darkening was observed in the urine of 2 subjects when the hydroxyphenyl excretion was minimal. Ascorbic acid given intramuscularly (500 mg./day) decreased the urinary excretion of tyrosine compounds although tyrosine feeding was continued.

Pteroylgintamic acid, 45 mg. intramuscularly given concurrently with tyrosine ingestion inhibited tyrosuria.

Vitamin B₁₂, 30 mcg. intramuscularly failed to influence the urinary excretion of tyrosine derivative whether given as a single injection during an established hydroxyphenyluria or twice a day concurrently with tyrosine feeding. Serum tyrosyl values were elevated during periods of tyrosuria. Serum amino nitrogen values paralleled the changes in serum tyrosyl content.

*Children's Memorial Hospital, and
Hoford Pharmacy
Toronto, Canada*

473. WADA, S., and ARAKAWA, T. *Blood picture of nutritional dystrophy in children: effect of folio acid and vitamin B₁₂ upon it (Studies on the nutrition of children in Hiroaki area, 10th report)* Tohoku J. Exper. Med. 56 47 June 25, 1952 (abstr. J. Am. Dietet. A. 29 64, Jan. 1953)

"Blood examinations were made of a group of Japanese children, who, though apparently healthy showed signs of the pellagrous form of nutritional dystrophy. The effects of folio acid and of vitamin B₁₂ upon the blood picture in these cases were also observed. Macrocytic anemia was found in 35 cases of the 46 examined in winter and in only 5 cases of the 33 examined in the summer. When 15 mg. folio acid were injected subcutaneously each day for two or three successive days, the majority of cases showed an increase in red blood cells, a reticulocyte response, a return to normal volume index, a decrease in white cells, and decreased eosinophil count, and a slight reduction in color index. The response to vitamin B₁₂ was minimal. Decreases in white cell count or in eosinophil count was observed in only a minority of cases treated with the vitamin."

GROWTH STUDIES

CHILDREN

474. WETZEL, N. C., FARGO, W. C., SMITH, I. H., and HELIKSON, J.: *Growth failure in school children as associated with vitamin B₁₂ deficiency—response to oral therapy*. *Science* 110: 651-653, Dec. 16, 1949.

The growth-promoting properties of vitamin B₁₂ have been demonstrated in certain bacteria and in animals. Since the dietary depletion method used in animals cannot be employed with children, it is necessary to observe the effects of vitamin B₁₂ under normal growth-promoting conditions in states of simple growth failure. These conditions include good hygiene, balanced exercise and rest, planned calorie conservation, emotional calm, and a satisfactory diet. One of the authors (Wetzel) had previously developed statistical methods by which the growth trends of individual children can be charted, and curves of expected growth plotted and compared with the actual subsequent growth. Wetzel's Grid is constituted of measures of various aspects of growth.

Vitamin supplements and whole liver extract given orally have frequently had beneficial effects in cases of simple growth failure in which delay in progress has been out of proportion to physical or other findings. However progress sometimes is unaccountably slow and this has suggested the existence of unknown deficiencies. In August 1949 the authors began administering 10 mcg. of crystalline vitamin B₁₂ each day orally to 11 children aged 5 to 12 years, 3 of whom were making slow progress and 8 of whom were selected at random from a larger group under regular care for varying degrees of malnutrition and in varying states of recovery from simple growth failure. All routine or special treatment a child had been receiving, such as extra rest or oral whole liver extract, was continued.

Five of the 11 children responded dramatically to this simple change in their routine, as shown statistically on the Wetzel Grid. Their growth responses over a period of eight weeks were equivalent to those to be expected from a further 100 to 240 days of regular institutional care without the help of B₁₂. In the authors' words, the results "speak with measurable statistical certainty of what may be termed B₁₂ functional deficiency that was definitely benefited by oral therapy."

The most dramatic general effects of vitamin B₁₂ were shown by a boy with severe allergic bronchitis, whose sleep had been interrupted regularly for 12 months by asthmatic attacks and whose desire and ability to eat had been greatly diminished by wheezing. The growth response in this case was accompanied by a remarkable attenuation of the asthmatic symptoms, which completely vanished during the first week of B₁₂ administration.

After B₁₂ administration the only noticeable clinical changes were increased physical vigor and alertness, better general behavior and greatly improved appetite. A moderate eosinophilia (6 to 8%) and reticulocytosis (0.6 to 0.9%) were found in 9 of the 11 children.

Children's French Air Camp and Hospital
Cleveland, Ohio

475. CHOW, B. F.: *Sequelae to the administration of vitamin B₁₂ to humans*, *J. Nutrition* 43: 323-343, Feb. 1951.

The effect of vitamin B₁₂ on the weight gain of chronically ill and clinically healthy children was studied. The chronically ill children were of various ages and had diseases such as rheumatic heart disease, malnutrition, anemia, and mental retardation. The normal children were 18 males ranging in age from 18 to 47 months. Both ill and normal children were given 25 mcg. of crystalline vitamin B₁₂ daily by mouth. Babies under 2 years of age received 10 mcg. of vitamin B₁₂ daily. Older children received tablets and younger ones, a solution of the vitamin in milk. Control children in the chronically ill group received no placebo (since they were already getting many pills) while healthy children serving as controls received a placebo solution in their milk. All children were weighed regularly.

In the chronically ill group, after three months of vitamin B₁₂ administration the mean gain in body weight in the experimental group was practically twice that in the control group. The difference in mean weight gains began to manifest itself after one month, and increased consistently with time. Among the normal children, the mean gain in body weight of the children in the B₁₂ group was consistently greater than that of the controls from the fourth week on. At the end of 24 weeks, the supplemented group showed a weight gain of 58 ounces, and the controls, a gain of 40 ounces.

However because of the heterogeneity of the ill children and because of the small number of normal children studied, the author considers the results as merely suggestive, although it is stated that the weight differences are statistically significant. The author states, however that "with an adequate diet, there is no reason to expect very dramatic effects from B₁₂ supplementation."

Oral administration of vitamin B₁₂ over a period of several months or in a single massive dose (5.0 mg. per adult) did not result in the appearance of vitamin B₁₂ activity in the urine. A major portion of the ingested vitamin B₁₂ appeared in the feces. These results indicate poor but apparently adequate absorption of vitamin B₁₂ from the gastrointestinal tract. When vitamin B₁₂ was given intravenously to man, the blood level decreased rapidly and the administered vitamin could be recovered in the urine. The vitamin was not absorbed by or did not penetrate into the erythrocytes in vivo or in vitro. When vitamin B₁₂ was given parenterally to infants fed on a soybean protein diet, no increase in nitrogen retention occurred.

Johns Hopkins University
Baltimore, Md.

476. BENJAMIN, B., and PIRRIE, G. D.: *Effect of vitamin B₁₂ on underweight children*, *Lancet* 1: 264, Feb. 2, 1952 (in Letters to the Editor).

An extensive and carefully controlled study by the London County Council failed to confirm the favorable results obtained by Wetzel (*Science* 110: 651, 1949 [Abstr. 474]) from the use of oral vitamin B₁₂ in underweight after long illness or from malnutrition.

dosage used was 10 mcg. daily of a product supplied by Glaxo. Vitamin B₁₂ affected neither growth nor the incidence of minor ailments.

London County Council
London, England

477. WEITZEL, N. C., HOPWOOD H. H., KUECHLE, M. E., and GRUENINGER, R. M. *Growth failure in school children. Further studies of vitamin B₁₂ dietary supplements*, J. Clin. Nutrition 1 17 Sept.-Oct. 1952 (abstr. J. Am. Dietet. A. 29 164 Feb. 1953)

"Group studies were designed to determine how effective vitamin B₁₂ supplementation for overcoming simple growth failure would be in the natural home environment. On the nutritional side, simple growth failure involves both substance as well as fuel debts which must be paid before recovery is achieved. Results of B₁₂ supplementation are shown in tables. Twenty children in one group received B₁₂ supplements for 16 weeks. In another group, 16 children were given the same supplement for 6 weeks. Of the total of 86 children, 23 responded definitely to the treatment by significant growth gain. No differences were found between crystalline and concentrated forms of the vitamin. From the standpoint of a nutrition program, the study reveals general benefits well beyond the point of individual recovery responses."

478. WEITZEL, N. C., HOPWOOD H. H., KUECHLE, M. E., and GRUENINGER, R. M. *Growth failure in school children further studies of vitamin B₁₂ dietary supplements*, J. Clin. Nutrition 1 17-31, Sept.-Oct. 1952.

Vitamin B₁₂ has been shown to exert a "growth promoting effect" when given to children in growth failure. From the regular public school enrollment lists of Shaker Heights, Ohio, children whose growth records showed a state of growth failure were selected for treatment and study. Oral vitamin B₁₂ supplements were given daily at the schools. (Double doses were given on Monday and Friday and none on Saturday and Sunday.) Dosage was 10 mcg. daily for 6 or 16 weeks. Sixteen of the 20 children in the 16-week program and 7 of the 16 in the 6-week program had an increased rate of growth after receiving the vitamin B₁₂ supplements. The net rate of gain for each group changed from below to above normal, showing that the children who responded were gaining part of the growth which should have been but was not gained in previous months. Other benefits accrued to the students, such as less fatigue, greater power of concentration, and better all-around progress than before.

In a further study involving 236 children and giving similar results, crystalline vitamin B₁₂ and concentrated vitamin B₁₂, both given orally were shown to be equivalent in growth-promoting effect.

Nutrition records of 45 children in the program showed that those who responded to vitamin B₁₂ supplements had a distinct advantage over the nonresponders in caloric intake, food choice and diet composition.

The growth pattern of one student is described in detail and her response to vitamin B₁₂ shown in the grid pattern.

Children's Fresh Air Camp and Hospital,
Shaker Heights Public Schools, and
Western Reserve University
Cleveland, Ohio

479. WILDE, E. *The treatment of growth failure in Ament school children*, J. Pediat. 40 565-569 May 1952.

A study was made, using the Wetzel Grid technique, of the effect of a proprietary nutritional supplement having B₁₂ activity (2.0 mcg./cc.) on the growth and development of 9 retarded school children, aged 10 to 14 years.

Wetzel Grid records were available for several years on these children and they were selected because of a slowdown in the rate of development. During a treatment period of seven months the nutritional supplement was given at school in doses of 5 cc. daily on the first four days and 10 cc. on the fifth. Since the children had previously received multivitamin tablets, it was felt that "improvement in the rate of growth and development in the treated children would probably be due to the added growth-stimulating effect of vitamin B₁₂."

The results are described as follows: "During the period of treatment there was a striking increase in the rate of growth in seven of the nine subjects, as measured in Grid levels per month. The growth rate in terms of developmental levels per month was restored, from a control rate of 0.71 before treatment to 1.02 levels per month. This is a value that coincides with the standard rate of 1 level per month characteristic of all healthy children of school age. In considering only the seven children who responded, the growth rate during the test period was 1.14 levels per month. This indicates that the growth failure had been stopped and that the previous lag was being corrected. The increase in the rate of growth and development is 65.2 per cent. These findings are consistent with the reports of Wetzel and associates, indicating that in some children growth failure occurs as a manifestation of functional deficiency of vitamin B₁₂ and that it may be corrected by oral administration of vitamin B₁₂."

St. Paul School
London

480. WILDE, E.: *The growth of Ament infants*, Canad. M. A. J. 68 70-71, Jan. 1953.

Daily administration of 1 cc. of a proprietary nutritional supplement increased the rate of growth, as measured by the Wetzel Grid, of 7 Ament infants who had been gaining normally before the experiment. The supplement contains, per cc., 0.8 mg. riboflavin, 5.0 mg. ferrous sulfate, and has the activity of 2.0 mcg. of vitamin B₁₂. Since prior to the experiment the infants had been given all these vitamins except vitamin B₁₂, the increase in growth rate must be attributed to the single new factor. The author regards it of interest that the growth-stimulating effect of such a small amount of vitamin B₁₂ (2.0 mcg. daily) can be detected by the Wetzel Grid.

Canada, Ont.

481. RESSA, E., and SORAGNI, E. *La vitamina B₁₂ quale fattore di accrescimento (Vitamin B₁₂ as growth factor)* Acta paediat. lat. 4 311-320, May June 1951

482. ROSSI, A. *La vit. B₁₂ in terapia quale fattore di sviluppo ed agente anabolizzante (Vitamin B₁₂ in therapy as a growth factor and anabolic agent)* Policl. Sez. prat. 58: 1099-1100, Aug. 27 1951
483. REVIEWS *Growth promotion by vitamin B₁₂ in children*, Nutrition Rev. 8 139-141 May 1950.
484. BIDAULT *Activité de la vitamine B₁₂ par voie orale chez l'enfant en que facteur de croissance (Activity of vitamin B₁₂ given orally as a growth factor in the child)* Concours méd. 73 3673-3674 Nov 3, 1951.
485. THERAPEUTICS *Hepatoterapia en el crecimiento infantil (Liver therapy in growth of children)* Orientacion Med. 1 602, April 17 1953.

Thirty-two children were given 1.20 Gm. of liver powder in tablet form daily for 13 weeks. 28 other children served as controls. There was a weight gain of 285 Gm. more and a 0.6 cm. greater increase in growth in the group that received liver than in the control group. It is stated that these results would indicate that the dietary factor which influences growth is liver and not vitamin B₁₂, as commonly believed.

INFANTS

486. SALMI, L. *Effect of vitamin B₁₂ in chronic nutritional disturbances in infants*, Clin. Pediatr. 32 617 Nov 1950 (abstr. A.M.A. Am. J. Dis. Child. 83 93, Jan. 1952)

"Five hypotrophic infants showed dramatic improvement clinically after the administration of vitamin B₁₂ (7 γ daily). Under this treatment the blood levels of protein, amino acids, iron, and cholesterol reached normal levels."

487. CASTLE, W. B., ELVEHJEM, C. A., MAY, C. D., WELCH, A. D., ZUELZER, W. W., and BUTLER, A. M. *Vitamin B₁₂ and folic acid in infant nutrition*, J.A.M.A. 146 1023-1029 July 14, 1951.

The adequacy of vitamin B₁₂ and of folic acid in milk diets customarily fed to infants was discussed in a report to the A.M.A. Council on Foods and Nutrition. Evidence was presented indicating that human milk contains between 0.1 and 1.0 mcg of vitamin B₁₂ and folic acid per liter. Cow's milk contains slightly more folic acid and definitely more vitamin B₁₂ than human milk. It was agreed that infants fed artificially with cow's milk formulae would receive as much vitamin B₁₂ and folic acid as breast fed infants, because these formulae usually contain 50 to 70 per cent cow's milk. Thus, there appears to be no need to supplement with vitamin B₁₂ and folic acid the diet of normal infants being fed cow's milk formulae.

The conference group did not believe that there was evidence of widespread deficiency of vitamin B₁₂ and folic acid among infants and children as revealed by megaloblastic anemia or suboptimal growth. A cautious attitude is assumed toward using vitamin B₁₂ or folic acid in an attempt to augment normal growth of healthy infants

being fed breast milk or cow's milk formulae. Certain pathologic conditions causing reduced intake or impaired assimilation in infants might increase the need of vitamin B₁₂ and folic acid. However the group did not feel that evidence presented justified widespread prophylactic use of vitamin B₁₂ or folic acid in order to meet the extra requirements of the occasional pathologic situation. A deficiency of ascorbic acid does cause an increased need of folic acid. Folic acid apparently serves as a precursor of the citrovorum factor and ascorbic acid enhances the production of this factor from folic acid. The evidence available does not indicate that vitamin B₁₂ is critically involved in the conversion of folic acid to citrovorum factor but it may be concerned with the subsequent formation, from this factor of another metabolically important compound.

488. MENEGHELLO J: *Progress of hospitalized malnourished infants*, J.A.M.A. 150 1505-1506, Dec. 13, 1952 (in Foreign Letters, Chile)

In a letter describing the treatment, mainly dietary of 1,539 malnourished hospitalized infants, it is mentioned that "To modify anemic conditions of the malnourished infants vitamin B₁₂ was given, producing a very satisfactory response in 75% of the cases."

489. BIDAULT G. *Activité de la vitamine B₁₂ par voie buccale, chez l'enfant en tant que facteur de croissance (Activity of vitamin B₁₂ given orally as a growth factor in the child)* Arch. Hospitalieres: 401-403 (No. 12) 1952.

Regardless of the cause and other treatment received, all undernourished children in the author's practice were given 50 mcg. of vitamin B₁₂ orally per day in two doses. This regimen was continued without interruption for 6 to 12 months, until "normal" values for height and weight were obtained. It was noted that in general weight gains began almost immediately while changes in height did not occur until after three months of therapy.

In the follow-up it was shown that when the children had been undernourished because of endocrine disease or infection, their growth rate had decreased so that three months after the last dose of vitamin B₁₂ they were on the verge of becoming undernourished for their age but not under weight for their size. When the original cause of small size was endocrine malfunction, the children had retained the gains made and were growing normally three or six months later.

It is suggested that vitamin B₁₂ can establish the endocrine equilibrium necessary for ideal growth, and is a useful adjunct to hormonal therapy (thyroid, somatotropin, testosterone, follicular hormone, androstenediol).

PREMATURE AND NEWBORN INFANTS

490. DOWNING, D. F. *Failure of vitamin B₁₂ to promote growth of premature infants*, Science 112 181 Aug. 11, 1950 (in Comments and Communications)

Of 48 premature infants with birth weights between 1,245 and 2,326 Gm., 25 were given vitamin B₁₂ intramuscularly in doses of 10 mcg. the other 23

controls. Frequency of administration varied from daily to every three days; the average total dose ranged from 30 to 220 mcg. Treatment was started on the second to seventh day of life.

Results were obtained which indicate that vitamin B₁₂ is ineffective in promoting weight gain of normal premature infants. It is suggested that vitamin B₁₂ administration will not benefit premature infant unless a specific deficiency of the vitamin exists.

The Pennsylvania Medical College and Hospital
Philadelphia, Pa.

- 491 RASCOFF H., DUNEWITZ, A., and NORTON R.: *The weight progress of premature infants given supplementary feedings of vitamin B₁₂, a comparative study* J. Pediat. 39 61-64, July 1951.

- 492 MITCHELL, T., ETTELDORF J. N., TUTTLE, A. H., and CLAYTON G. W. *Crystalline vitamin B₁₂ and sodium folate in prematurity* Pediatrics 18 821-827 Dec. 1951

Crystalline vitamin B₁₂ and sodium folate, separately or together were given intramuscularly to 52 premature babies. Records of weight, length, hemoglobin, hematocrit, erythrocyte count, reticulocyte count, and calculations of the mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume failed to show any influence of these vitamins, singly or in combination during the first 50 days

of life, on these infants as compared with 24 premature infants who did not receive the vitamins.

University of Tennessee College of Medicine, and
John Cotton Hospital
Memphis, Tenn.

- 493 PARADISO M., and MONDANI E. *Action of vitamin B₁₂ on increase of immaturity* Riv. clin. pediat. 49 590-1951 (abstr. A.M.A. Am. J. Dis. Child. 83 523, April 1952)

"The authors tested the action of vitamin B₁₂ in premature infants and noted its therapeutic efficacy thus confirming the results of experimental researches made by others on animals. The authors consider the possible mechanism of action of the vitamin."

- 494 CHINNOCK, R. F., and ROSENBERG H. W. *Results of administration of vitamin B₁₂ to newborn infants*, J. Pediat. 40 182-185, Feb. 1952.

The effect of oral vitamin B₁₂ on weight gain and growth in height was studied in full-term infants. The daily dose of vitamin B₁₂ was 5 mcg., and was added to the milk once daily. Twelve infants received the vitamin for the first 60 days of life, and 11 received it for 30 days. 6 served as controls and were given a placebo. No difference in weight gain or increase in length was noted in infants receiving vitamin B₁₂ as compared with the controls.

College of Medical Surgeons
Los Angeles, Calif.

ANALYTICAL STUDIES

VITAMIN B₁₂ ACTIVITY IN THE BODY

- 495 GRANADOS, H., GLAVIND J., NOER, B., and DAM, H.: *Studies on the growth-promoting action of human saliva*, Acta path. et microbiol. Scand. 27 501-505, 1950.

Concentration of vitamin B₁₂ in human saliva, determined by a microbiological method, was found to be 0.00015 to 0.0005 micrograms per milliliter

- 496 TOMASZEWSKI, W. *The fluorescence phenomenon of the tongue*, Brit. M. J. 1 117-120, Jan. 20, 1951.

When the mouth is viewed under ultraviolet illumination screened with Wood's glass a reddish-orange fluorescence is often visible on the dorsum of the tongue and sometimes on the teeth. This fluorescence is due to the production of porphyrins by oral microorganisms.

The author has examined more than 400 patients for fluorescence of the surface of the tongue and teeth. These patients had various diseases, some characterized by changes in the tongue. More than 200 healthy persons were also examined for control purposes. In some cases, antibiotics, vitamins, and chemotherapeutic agents were given in order to investigate the influence of these substances in the fluorescence.

It was found that fluorescence was absent more frequently with increasing age, being absent in only 11 per cent of children and in 53 per cent of people over 80

years of age. Fluorescence is absent more frequently in certain diseases, such as pernicious anemia, hypochromic anemia, sprue syndrome, and vitamin B₁₂ deficiencies than in the healthy state. When vitamins of the B group, including vitamin B₁₂, folic acid and riboflavin, are administered, fluorescence is restored and/or intensified in a large percentage of cases. Antibiotics destroy fluorescence by their action on microflora.

The fluorescence phenomenon seems to be dependent on two factors: the presence of porphyrin-producing bacteria, and the presence of normal papillae on the tongue. Its clinical significance is not known. It may be a guide to the state of nutrition in the individual.

Royal Infirmary
Edinburgh, Scotland

- 497 ROSS, G. I. M. *Vitamin B₁₂ assay in body fluids*, Nature 166 270-271 Aug. 12, 1950 (Letter to Editors)

The writer describes a modification of Hutner's technique for the assay of vitamin B₁₂ in body fluids, using the alga *Engelmannia gracilis* variant *bacillaris*. This method is more sensitive than the *Lactobacillus* method.

Postgraduate Medical School of London
London, England

- 498 ROSS, G. I. M.: *Vitamin B₁₂ assay in body fluids using Engelmannia gracilis*, J. Clin. Path. 5 250-Aug. 1952.

- 499 ROSENTHAL, H. L., and SARETT, H. P.: *The determination of vitamin B₁₂ activity in human serum*, J. Biol. Chem. 199 433- Nov 1952 (abstr. J. Am. Dietet. A. 29 162, Feb. 1953)

Preliminary treatment of human serum necessary for microbiological determination of vitamin B₁₂ activity with *Lactobacillus leichmannii* was studied with samples of serum in the postabsorptive state. The results indicate that the vitamin B₁₂-active material in serum is firmly bound and is not readily released by dialysis but is liberated by heating under suitable conditions."

500. MOLLIN D. L., and ROSS G. I. M.: *Vitamin B₁₂ in practice*, Lancet 1 46, Jan. 8, 1953.

The authors state that patients with vitamin B₁₂ deficiency can be separated from those with folic acid deficiency by microbiological assay of vitamin B₁₂ in the serum, using *Engelmannia gracilis* var. *bailliaris*. Serum from patients with vitamin B₁₂ deficiency shows a low vitamin B₁₂ content, usually below 50 micromicrograms per cc. Serum from normal subjects and from patients with folic acid deficiency always has an assay of vitamin B₁₂ above 100 micromicrograms per cc.

Occasional false high assays are encountered, but if more than one pre-treatment serum sample is tested, "the method becomes very reliable, and a definitely low serum B₁₂ concentration can be confidently accepted as proof of severe vitamin B₁₂ deficiency in the body."

Pregraduate Medical School of London
London, England

METABOLIC PROCESSES

501. JUTTON, D. R., and PARSONS, H. T.: *Excretion of vitamin B₁₂ by human subjects*, Federation Proc. 10 203, March 1951.

"Intramuscular injection of 450 mg. vitamin B₁₂ for 4 subjects (women) led to a urinary return of 37-50% of the dose within 6 hours and 40-75% within 12 hours, in agreement with reports of others on men. In agreement also was the almost complete failure to find vitamin B₁₂ activity in the urine on normal diets or after oral dosage. After parenteral administration, a small amount of microbiological activity for *Lactobacillus leichmannii* 4797 (A.T.C.C.) was detected in the samples of bile-rich duodenal contents within 30 minutes, while the basal specimens showed none. Observations on the vitamin B₁₂ activity of the feces of these subjects gave no clear evidence of increases following dosage."

University of Minnesota
Minneapolis, Minn.

502. REGISTER, U. D., and SARETT, H. P.: *Urinary excretion of vitamin B₁₂, folic acid and citrovorum factor in human subjects on various diets*, Proc. Soc. Exper. Biol. & Med. 77 837-839 Aug. 1951.

Recent reports have suggested that the urine of normal subjects has no appreciable vitamin B₁₂ activity. In the present study small but measurable amounts of vitamin B₁₂ were found in the urines of 56 medical students on various diets. In 14 subjects on normal diets, the urinary excretion of vitamin B₁₂ averaged 81 my

(millimicrogram) a day. Similar values were found in 28 subjects maintained for three days on milk, alkaline ash, low protein, or high protein diets. A somewhat higher value was found in 11 subjects on high purine diets, and the increase over normal was shown to be due to interfering compounds. On the third days of fasting, 6 subjects were found to excrete an average of 62 my of vitamin B₁₂ a day. The average citrovorum factor (CF) excretion by the subjects on these diets accounted for only part of the total folic acid (FA) compounds excreted and appeared to be correlated with the total FA values. Differences in vitamin B₁₂ excretion resulting from dietary changes were not similar to those observed in CF and FA excretion.

Johns Hopkins School of Medicine
New Orleans, La.

503. LANG C., HARTE, R. A., CONLEY C. L., and CHOW B. F.: *Retention of crystalline vitamin B₁₂ by healthy male individuals following intramuscular injection*, J. Nutrition 46 215-221 Feb. 11, 1952.

Authors summary "Crystalline vitamin B₁₂ was administered intramuscularly to groups of healthy male subjects at the following levels 20, 50, and 75 µg. Urinary collections from each subject were made during 24 hours after injection, each in two fractions representing the first 8 hours and the following 16 hours. The vitamin B₁₂ microbiological activities in the urine specimens were measured. These data permitted calculation of the amount retained, by taking the difference between the injected and the excreted vitamin. It was found that more than 50 µg. of vitamin were retained daily by these subjects, depending on the dosage administered."

The Johns Hopkins Hospital
Baltimore, Md.
Sharp and Doherty Inst.
Columbia, Pa.

- 504 WATKIN D. M., LANG C. A., SHOCK, N. W., and CHOW B. F.: *Age-wise differences in urinary excretion of vitamin B₁₂ following intramuscular administration*, Federation Proc. 12 151 March 1953.

"The urinary excretion of vitamin B₁₂ in 24 hours following its intramuscular administration in crystalline form was studied at different dose levels in 107 male subjects in widely separated age categories. The results of two separate studies [summarized in tabular form] demonstrate that B₁₂ excretion assayed microbiologically diminished in an orderly and significant fashion with age. Significance was tested at the 0.05 level by an analysis of variance. Decrements in B₁₂ excretion with age paralleled previously observed decrements in renal function with age. However the observed differences cannot, on the basis of data arising from these studies, be attributed to renal factors alone."

National Heart Institute
Bethesda, Md.
Johns Hopkins School of Hygiene and Public Health
Baltimore, Md.

505. LANG C. A., BECKER, B., GLEYSTEN D., and CHOW B. F.: *Diabetic retinopathy and urinary excretion of vitamin B₁₂*, Federation Proc. 12 421, March 1953.

"The marked retention of radioactivity by the pancreas following injection of radiolabeled vitamin B₁₂ and its involvement in carbohydrate metabolism suggest a possible role of vitamin B₁₂ in pancreatic diseases such as diabetes mellitus, which might manifest itself in an abnormal excretion of a test dose. The results of an initial experiment indicated a correlation in the urinary excretion of administered B₁₂ and diabetic retinopathy. To examine this hypothesis, consecutive diabetic patients seen at The Wilmer Institute regardless of age, sex, or other diseases were injected intramuscularly with 50 mcg. crystalline vitamin B₁₂. These patients were classified as to the presence or absence of retinopathy. Urine specimens collected prior to and from 0-8 hours after administration were assayed for the total microbial activity of B₁₂. To avoid bias the diagnoses and B₁₂ analyses were not correlated until the final assay results were obtained. In this series of 35 diabetic the mean urinary excretions of B₁₂ were 4.2 ± 1.7 mcg. for the 13 subjects without retinopathy and 19 ± 2.1 mcg. for the 22 subjects with retinopathy. The latter value was statistically higher than that (9.6 ± 1.4) of healthy subjects in a comparable age range. Only two individuals, both in the non retinopathy group excreted amounts overlapping into the opposing group. Although subsequent examination disclosed the presence of retinal disease, they were not removed from their original group for statistical analyses. Even then a significant difference ($P < 0.001$) between the two diabetic groups was obtained. The lower B₁₂ activity in the urine could be due to the excretion of metabolically inactivated B₁₂, the presence of inhibitors, or a greater retention of the vitamin. However when B₁₂ containing radioactive Co⁶⁰ was injected into several subjects in both diabetic groups, a direct correlation of radioactivity and microbial activity was found in the urine. These results thus favor the last hypothesis."

The Johns Hopkins University
Baltimore, Md.

506. UNGLAUB, W. G., ROSENTHAL, H. L., and GOLDSMITH, G. A. *Vitamin B₁₂ in blood and urine after oral and parenteral administration*, Federation Proc. 12 432, March 1953.

"Following intramuscular administration of 10 to 100 µg. of vitamin B₁₂ to normal subjects, serum vitamin B₁₂ activity rose in one hour to maximum values which were proportional to the amounts injected. Urinary vitamin B₁₂ activity in 24 hr. following injection increases with the quantity administered, but cannot be predicted accurately. Serum vitamin B₁₂ activity during fasting is consistently lower in patients with untreated macrocytic anemia than in normal subjects. In 2 patients with pernicious anemia in remission who received 25 µg. of vitamin B₁₂ intramuscularly increases in serum activity did not differ from those of normal subjects but vitamin B₁₂ activity in urine was somewhat less than normal. Serum vitamin B₁₂ activity did not increase significantly when normal subjects were given 500 and 1000 µg. orally. After oral administration of 5000 µg., serum activity reached maximum values equal to those found after intramuscular injection of 10-25 µg. Serum activity remained elevated for longer periods after oral than after intramuscular administration. In 3 of 4 patients with macrocytic anemia who received 5000 µg. of vitamin B₁₂ orally

serum activity increased to maximum value similar to those noted after intramuscular injection of 1-50 µg. In the 4th no significant rise occurred. In the 5th patient serum activity increased after oral administration of 500 and 1000 µg. Optimal hematologic response resulted in all patients. Following oral doses, no significant increase in vitamin B₁₂ activity was found in the urine."

Yale University School of Medicine
New Haven, Ct.

507. STUHLFAUTH, K. *Influence of vitamin B₁₂ on levulose content of blood*, Klin. Wchnschr. 31 312 April 1 1953 (abstr. J.A.M.A. 152 1882 1953, Aug. 1, 1953)

"Stahlitz reported in 1937 that the increased blood levulose concentration that results from the administration of levulose can be reduced by administration of liver extract. Stuhlfauth noted that the same effect could be obtained when, instead of liver extract, he administered from 15 to 30 µg. of vitamin B₁₂ intramuscularly after levulose had been given either intravenously or by intraduodenal drip. Stuhlfauth was interested in ascertaining whether this reduction in the blood levulose content, which he had obtained in 30 subjects, including some with hepatic disorders and with obesity would also influence other constituents of the carbohydrate metabolism. For this purpose levulose was introduced into the diet of 10 fasting persons. When 1 liter of 5% levulose solution was given hourly the blood levulose concentration was found to be between 30 and 50 mg. per 100 cc. after about 120 minutes and remained at this level when the levulose administration was continued at the same rate. If, after the levulose content has reached its maximum, the patient is given 30 µg. of vitamin B₁₂, the levulose nearly always decreases by from 10 to 25 mg. per 100 cc. The blood dextrose then increases slightly.

Lactic acid usually increases noticeably sometimes to twice its original value, one hour after injection of vitamin B₁₂. The pyruvic acid decreases following a slight temporary increase. The inorganic phosphate one hour following the injection of vitamin B₁₂ shows a renewed increase, which is followed by a temporary decrease. The reduction in the blood levulose content occurs only after injection of vitamin B₁₂ or of liver extracts. Cobalt, folic acid, and adrenal cortex extract did not have this effect on the levulose content of the blood. Administration of 30 µg. of vitamin B₁₂ to eight fasting healthy persons who had not been given levulose likewise showed a slight decrease in the general function of reduction. In five patients with diabetes no uniform changes could be observed. The author concludes that the changes appearing simultaneously in the serum contents of dextrose, pyruvic acid, and inorganic phosphate indicate that vitamin B₁₂ induces increased utilization of levulose."

NUTRITION IN GENERAL

508. KING, C. G. *New advances in the science of nutrition*, J. Am. Dietet. A. 23 109-111, Feb 1949

Nutrition Foundation, Inc.
New York, N. Y.

509. SPIES, T. D. *Recent progress in nutrition*, Post grad. Med. 6 96-106, Aug. 1949

A number of subjects are briefly discussed in this paper. One emaciated patient with nutritional macrocytic anemia responded well to folic acid. Charts and photographs show his improvement under adequate treatment. A patient with sprue also made a good response to folic acid. Lateral sclerosis in pernicious anemia, however, is not prevented or relieved by folic acid. In this condition vitamin B₁₂ is effective. When given intramuscularly only very small doses are required: 8 micrograms in tropical and non-tropical sprue, and 15 micrograms in pernicious anemia. Intravenous administration has been used in one series of patients and subcutaneous in another and both methods have proved effective and non-toxic. When vitamin B₁₂ is given orally about 50 times as much is required to produce a given blood response as when it is given intramuscularly. The oral dose can be reduced to one-fifth or one-tenth, however, if the vitamin is incubated overnight with normal human gastric juice.

Similar results have been obtained with vitamin B₁₂ in nutritional macrocytic anemia and in pernicious anemia. In 2 patients, symptoms of very acute combined system disease disappeared following the oral administration of 1.5 mg. of vitamin B₁₂ over a period of 10 days. Massive doses are necessary in this condition, but the results depend more on the chronicity of the disease than on the extent of the neurologic involvement. The most dramatic responses have occurred in patients with symptoms of less than a month's duration.

William Hooper
Birmingham, Ala.

510. HAUSMANN K. *Liver extracts, vitamin B₁₂, and thymidine*, Lancet 2 962-963, Nov 19 1949 (in Letters to the Editor)

The writer assumes the possible existence of at least three groups of different forms of vitamin B₁₂: 1) cobalt-containing red compounds which on intramuscular injection, even in high doses, are ineffective in pernicious anemia but which with enzymatic cleavage become heme topologically active; 2) cobalt-containing red pigments that are clinically active and precipitable by ammonium sulfate, as contained in purified liver extracts and described by Lester Smith; 3) cobalt-containing red pigments, clinically active but soluble in ammonium sulfate, which are present especially in autolyzed liver and in urine. It is suggested that under the influence of certain enzymes, which the writer proposes to designate as vitamin B₁₂ conjugases and which are to be found in stomach mucosa, pancreas, kidneys, and presumably also in liver papain, moulds, and bacteria, the different forms of vitamin B₁₂ may become transformed one into the other and vary in the liver according to the degree of autolysis.

St. Georg General Hospital
Hamburg, Germany

511. MUSHETT C. W. *Vitamin B₁₂—A nutritional factor for man and beast*, Am. Biol. Teacher 11 134-141, Oct. 1950.
512. SPIES T. D. *Recent advances in diagnosis and treatment of deficiency diseases*, J.A.M.A. 143 66-72, Jan. 13, 1951.

Difficulties in diagnosing conditions of undernutrition and malnutrition are discussed and illustrative case histories are presented.

The author details his current method of giving vitamins orally to patients with signs of nutritional deficiency (including the clinical syndromes of sprue, beriberi, pellagra, riboflavin deficiency, scurvy and certain macrocytic anemias). The following daily doses of vitamins are given: folic acid, 5 mg.; thiamine, 10 mg.; nicotinamide, 150 mg.; riboflavin, 10 mg.; ascorbic acid, 150 mg.; and vitamin B₁₂, 10 mcg.

Experiences with folic acid and vitamin B₁₂ in the treatment of anemias are reviewed. Dosage of vitamin B₁₂, both oral and parenteral, is discussed. The minimum effective parenteral dose is 1 mcg. daily and the effective oral dose in most patients is 30 to 60 times the parenteral dose. The author's studies to date show no demonstrable difference in activity per unit of weight for vitamin B₁₂, vitamin B_{12a}, and vitamin B_{12b}, the last two being identical substances. It is stated that concentrates of vitamin B₁₂ seem to be about as well tolerated as solutions [of the crystalline preparation?]

Northwestern University Medical School
Chicago, Ill.

513. REVIEW: *Festival symposium on nutrition and the B vitamins*, Brit. M. J. 2 49-51 July 7 1951.

This is a report of the speeches made at the symposium. Subjects discussed were vitamin B₁ deficiency in man, nature and occurrence of vitamin B₁₂, treatment of megaloblastic anemia with vitamin B₁₂, malnutrition in West Africa, and pellagra.

514. GOLDSMITH, G. A., and GIBBENS J.: *Recent advances in nutrition review of the literature 1949-1950*, A.M.A. Arch. Int. Med. 88 93-131 July 1951.

In the section of this review devoted to vitamins, subjects discussed are: folic acid, citrovorum factor vitamin B₁₂ and choline in liver disease; vitamin A, folic acid, vitamin B₁₂, choline, inositol, thiamine, riboflavin, pyridoxine, vitamin B complex, ascorbic acid, vitamin D vitamin E, and vitamin K.

Tulane University School of Medicine
New Orleans, La.

515. SPIES T. D., STONE, R. E., GARCIA LOPEZ, G., LOPEZ TOCA, R., and REBOREDO A. *Therapeutic indications for vitamins in mixtures* Post grad. Med. 10 269-284 Oct. 1951.

A patient who had a low plasma ascorbic acid level developed areas of hemorrhage around hair follicles on the leg. The hemorrhagic areas faded within 72 hours after the daily administration of 500 mg. of synthetic ascorbic acid was begun. (Rats fed on the diet used by the patient did poorly. Supplementation with the B vitamins improved their condition, and liver extract was still more effective.) It was expected that the patient would develop symptoms of other deficiency diseases, and eventually she did develop a macrocytic anemia. Administration of folic acid resulted in striking improvement. Glossitis developed and was relieved by vitamin B₁₂.

a day. Later edema developed and a diagnosis of beriberi was considered. This condition was relieved by this mine treatment.

Vitamin B₁₂ was effective in a case of acute combined degeneration of the spinal cord. A patient with pernicious anemia responded at different times to a number of agents—folic acid, 5-methyl uracil, vitamin B₁₂, vitamin B_{12a}, and B_{12c}, and folic acid. A patient with pellagra responded to folic acid, and later her nursing child was successfully treated for anemia with folic acid followed by a single injection of vitamin B₁₂. A patient with macrocytic anemia of pregnancy failed to respond to vitamin B₁₂ intramuscularly or orally but responded to oral administration of vitamin B₁₂ combined with folic acid. The diarrhea associated with tropical sprue may be relieved by either vitamin B₁₂ or folic acid, and sometimes (but rarely) the combination of the two is more effective than either alone. Illustrations are presented of a child with chlorosis due to riboflavin deficiency and one with rickets.

Dietaries of families with nutritional deficiency diseases are discussed, and dietary and banded therapy are outlined. Methods of administration of the vitamins are discussed. Basic vitamin therapy includes thiamine, riboflavin, niacinamide, ascorbic acid, folic acid orally and vitamin B₁₂ when it can be absorbed. In addition, synthetic vitamins may be given orally or parenterally and natural vitamin complex in the form of brewer's yeast or extract and/or liver extract may be given orally or parenterally.

*Yale University School of Medicine
Chicago, Ill.
Fulton Hospital
Birmingham, Ala.
General Clinical Center Hospital
Kansas, Kans.*

516. TOMARELLI, R. M., LINDEN, E., and BERNHART, F. W.: *Nutritional quality of milk thermally modified to reduce allergic reaction*, *Pediatr* 19: 89-93, Jan. 1952.

Infants and children allergic to cow's milk, can take milk which has been made hypoallergenic by prolonged heat treatment, but experiments with rats have shown that milk thus treated is reduced in nutritional quality. Fortification of heat-treated milk with minerals and all the usual vitamins with the exception of vitamin B₁₂ did not restore its nutritional value for young rats, but addition of liver powder or of a vitamin mixture containing vitamin B₁₂ resulted in a growth rate equal to that of rats fed untreated milk. The addition of aureomycin to the heat-treated milk, with or without vitamin B₁₂, did not increase the growth rate.

Yale University School of Medicine, New Haven, Conn.

517. CHOW, B. F.: *The role of vitamin B₁₂ in metabolism*, *South. M. J.* 45: 604-612, July 1952.

Pharmacologic and metabolic studies in the literature are reviewed, and the author gives the results of his own investigations. Urinary and fecal excretion of vitamin B₁₂ and the content of the vitamin in kidney, liver and intestines after administration were studied. Nitrogen retention in infants appeared not to be affected by vitamin B₁₂ supplementation. Children with chronic diseases

(stress) appeared to gain more weight and to gain it more rapidly when they were given 25 mcg. of vitamin B₁₂ daily by mouth. In healthy children the results of such supplementation were equivocal. Vitamin B₁₂ appears to play no direct part in protein metabolism but it is apparently involved in carbohydrate and/or fat metabolism. Its effect on weight gain in young growing female rats is greater than in littermate males when the major source of calories is carbohydrate, and is greater when the diet is high in carbohydrate than when it is high in fat or in fat-carbohydrate mixtures.

*Johns Hopkins University
Baltimore, Md.*

518. SURE, B.: *Improvement in protein efficiency of a whole wheat cereal breakfast food with the amino acids lysine and threonine and vitamin B₁₂*, *Arch. Pediatr.* 69: 859. Sept. 1952 (abstr. *J. Am. Dietet. A.* 29: 56, Jan. 1953).

"Enrichment of cereal grains promises a more nutritious diet for large portions of the world's population who cannot afford to supplement their diets with a sufficient quantity of the important animal products, and for whom cereals are the basic food. An investigation of the value of the protein enrichment of cereals was made on albino rats. The rations consisted of a processed whole wheat cereal breakfast food plus supplements of vitamin B₁₂, lysine, valine, threonine, and a fish-soluble extract in various combinations. The B₁₂ supplement proved of no value in increasing growth, but when combined with the fish-soluble extract or with the three amino acids resulted in remarkable growth."

519. GOLDSMITH, G. A., UNGLAUB, W. G., and GIBBENS, J.: *Recent advances in nutrition and metabolism: review of the literature, 1951*, *A.M.A. Arch. Int. Med.* 90: 518-561, Oct. 1952.

This review covers 850 references, most of them multiple, to nutrition and metabolism. The subjects reviewed include caloric nutrition, fat metabolism, nutrition and hypertension, protein nutrition, carbohydrate nutrition, vitamins (A, thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid, citrovorum factor B₁₂, choline, inositol, ascorbic acid, D, E, and K) and mineral nutrition. Sprue, and nutrition in liver disease are discussed.

*Yale University School of Medicine
New Haven, Conn.*

520. COWGILL, G. R.: *Recent trends in nutrition and future possibilities*, *Connecticut M. J.* 17: 91-97 Feb. 1953.

Nutritional studies during the first half of the century have shown that the energy or caloric factor, the protein factor, certain mineral or inorganic nutrients and vitamins are essential.

There is some evidence that the caloric requirements calculated from surveys made at the turn of the century are now too high since muscular activity has decreased during this period.

Study of protein has shown that 10 amino acids are essential for the growth of the rat, and 11 for maintenance

of nitrogen equilibrium in man. It is now recognized that proteins are as important nutritionally as vitamins.

Many antivitamins have been discovered which can replace the natural vitamin for the first steps in a sequence of chemical changes but cannot complete the sequence. This may be advantageous if it interferes with the growth of disease bacteria which depend on a certain vitamin, or it may produce symptoms of vitamin deficiency. Recently pyridoxine deficiency has been produced for the first time in human volunteers by the use of the antivitamin desoxy pyridoxine, showing that pyridoxine is essential for man.

The history of the development of vitamin B₁₂ and its effect in pernicious anemia and in promoting growth are discussed.

Studies in progress at the Institute of Nutrition of Central America and Panama (INCAP) suggest that humans require a small amount of animal protein and that supplements of B₁₂ and/or certain antibiotics (penicillin, aureomycin) may promote growth in children who receive little or no animal protein, and may improve plant food diets.

Low sodium diets are discussed and the danger of too great depletion of other minerals, protein and B-complex vitamins by means of such diets is pointed out. Informative labeling of food products processed to reduce sodium content is urged.

Another topic discussed is the effect of fats and cholesterol on hardening of the arteries.

Methods of improving nutrition by improving the quality of foods include the enrichment of wheat flour of rice (to prevent beriberi) and of corn meal (to prevent pellagra). Another means of preventing pellagra is to encourage the use of milk, since milk contains tryptophan and this amino acid is used by the body in manufacturing nicotinic acid, the antipellagra vitamin.

Quality of foods might further be improved by genetic selection of varieties of plants which have high content of vitamins (thiamine, niacin, ascorbic acid).

*Yale University
New Haven, Conn.*

- 521 ELVEHJEM, C. A. *Value of natural foods*, J. Am. Dietet. A. 29 178, Feb. 1953.

In an address before the Nutrition Foundation, the following reference to vitamin B₁₂ was included.

"Since we now know that most of the vitamins function by supplying the prosthetic group or the working end of an enzyme, it is not surprising to find some relation between amino acids needed for building protein and a vitamin. Recently it has been found that vitamin B₁₂ protects the young rat on a diet low in choline and methionine against a fatty liver and also against kidney damage. Work in our laboratory using a low-protein diet of 9 per cent casein has shown that in the absence of choline, B₁₂ has little, if any protective action on the production of fatty livers. This undoubtedly means that with no choline and a low supply of methionine, vitamin B₁₂ is unable to overcome any of the deficiency. When small limiting levels of choline and a little higher levels of methionine are used, the vitamin B₁₂ does have some

sparing effect. We have recently found that even in the presence of adequate methionine, choline, and vitamin B₁₂, the fat content of livers is not normal when a low protein diet is fed. Certain other amino acids are needed to bring the fat content of the liver to a normal level. Furthermore, some of these changes can be aggravated by an imbalance of certain amino acids. This emphasizes the importance of supplying amino acids in foods rather than in combinations which might be quite different from those usually encountered in food materials."

*University of Wisconsin
Madison, Wis.*

522. MICKELSEN O.: *Nutritional aspects of antibiotics*, J. Am. Dietet. A. 29 221-229 March 1953.

The literature on the increase in growth of animals when antibiotics are added to commercial rations is reviewed. Whether the increase is the result of a sparing action on vitamin and protein requirements, favoring the growth of microorganisms which synthesize some unknown growth factor decreasing the growth of bacteria which would use large amounts of some essential dietary nutrient, inhibition of the growth of microorganisms which might produce disease, or favoring the absorption of nutrients from the gastrointestinal tract is not yet known. The increase in the rate of growth of animals may lead to cheaper and more abundant supplies of meat for human consumption.

Aureomycin has been found to produce improvement in clinical cases of pernicious anemia, perhaps by lessening the patient's vitamin B₁₂ requirement, probably by increasing the synthesis of this vitamin in the gastrointestinal tract. Improvement of megaloblastic anemia of pregnancy under penicillin treatment has also been reported. Porriani (*Boll. Soc. Ital. Biol. Sper.* 27: 1151 1951 *Chem. Abstr.* 46: 5192, 1951) has reported increase in weight gain in infants given aureomycin, as has Robinson (*Lancet* 1: 52, 1952).

Studies are being conducted by the Institute of Nutrition of Central America and Panama (*Proc. Food and Nutrition Board* 12: 21, May 1952) on the effect of supplementing the diets of school children with skim milk, vitamin B₁₂ (20 mcg. a day) or aureomycin (50 mg. a day). In children receiving only 5 Gm. of animal protein a day skim milk slightly increased growth, vitamin B₁₂ was considerably more effective, and aureomycin produced a growth rate almost twice that of children receiving placebo. In children receiving 18 Gm. of animal protein a day neither vitamin B₁₂ nor aureomycin increased the growth rate. The possibility of improving, by the addition of B₁₂ or antibiotics to the diet, the nutritional status of the peoples who receive little animal protein is suggested. In this country antibiotic supplementation would probably not be dramatic since the usual intake of animal protein is fairly high.

*Medical Department of Health
Northridge, N.Y.*

523. SEHRELL, W. H., Jr.: *Trends and needs in nutrition*, J.A.M.A. 152 42-44, May 2, 1953.

In this discussion of the present status and the future of nutrition as it relates to the field of medicine, the following excerpts deal with the place of vitamin B

the total nutritional picture. "Interrelations have been established between choline and the vitamins B₁₂, folic acid, and ascorbic acid and these have been related to the amino acids cystine, methionine, tyrosine, and phenylalanine.

"In other studies continued attempts have been made to clarify the mechanism through which a lack of choline may damage the kidneys and liver. Absence of this vitamin from the diet has killed rats within five days. The specific effect, apparently vascular, can be minimized by several procedures, including administration of vitamin

B₁₂, corticotropin, or nonspecific stimuli including an alarm reaction."

In summary the author states: "A reoriented nutrition program in this country is much needed, with increased emphasis on clinical research aimed primarily at obesity chronic diseases, and borderline deficiencies. In addition, there is need for intensified research on the special nutritional requirements of the aged and persons under stress."

National Institutes of Health
Bethesda, Md.

ANIMAL STUDIES

FERTILITY STUDIES

Chicks

- 524 LILLIE, R. J., OLSEN M. W., and BIRD H. R.: Role of vitamin B₁₂ in reproduction of poultry. Proc. Soc. Exper. Biol. & Med. 72 598-602, Dec. 1949

During the incubation period eggs laid by Rhode Island Red hens fed a diet deficient in vitamin B₁₂ were injected with 0.5 to 1.25 mcg. of vitamin B₁₂ per egg in aqueous solution. The rate of hatchability of these eggs was greater than that of eggs injected with sterile water or not injected. In the chicks from the eggs injected with vitamin B₁₂ the mortality was lower the growth rate was greater and the feathering better than in chicks hatched from eggs injected with water or not injected. Adding vitamin B₁₂ to the diet of chicks hatched from vitamin B₁₂ deficient eggs increased the growth response but did not reduce the mortality nor improve the feathering. This emphasizes the importance of the maternal diet, which, if deficient, may lead to irretrievable postnatal damage as well as prenatal effects. The greater growth response occurred from a combination of dietary supplement with egg injection.

U. S. Department of Agriculture
Beltsville, Md.

525. OLCESE, O., COUCH, J. R., and LYMAN C. M. Vitamin B₁₂ concentrates in the nutrition of the mature domestic fowl, J. Nutrition 41 73-87 May 1950.

Authors summary "When a sucrose-soybean protein diet, low in vitamin B₁₂, was fed to a group of hens, egg production decreased. Hatchability of eggs from hens fed this low vitamin B₁₂ diet decreased to zero in from 8 to 6 weeks. The addition of APF concentrates improved the egg production and hatchability of hens fed the purified diet but failed to produce normal hatchability as compared to a group fed a practical all-mash diet. When a starch-soybean protein diet was used, egg production and hatchability were improved over those observed with comparable groups fed sucrose as a source of carbohydrate, indicating that starch prompted the intestinal synthesis of vitamin B₁₂ and possibly some unknown factor required for embryonic development in the fowl. The hatchability of eggs from hens receiving the purified diets showed a tendency to decrease about the 9th or 10th experimental week, even though adequate amounts of

vitamin B₁₂ were fed. This indicates that a depletion of some unknown factor or factors may have taken place after the initial depletion of vitamin B₁₂."

Agricultural and Mechanical College of Texas
College Station, Texas

526. OLCESE, O., COUCH, J. R., QUISENBERRY H. H., and PEARSON P. B.: Congenital anomalies in the chick due to vitamin B₁₂ deficiency J. Nutrition 41 423-431, July 1950.

A definite peak of embryonic mortality occurred at the 17th day of incubation in eggs from hens fed a diet deficient in vitamin B₁₂. The most characteristic symptom of deficiency present in the embryos was myostrophy of the leg. A malposition, "head between the thighs," occurred with unusual frequency in eggs from hens fed a vitamin B₁₂-deficient diet. Other anomalies associated with vitamin B₁₂ deficiency in this study were hemorrhages of the embryo and allantois, and periosis.

- 527 KLINE, I. T., and DORFMAN R. L. Estrogen stimulation of the oviduct in vitamin-deficient chicks, Endocrinology 48 345-357 April 1951.

The relation of single vitamin deficiencies to the action of stilbestrol on the oviduct of the chick was studied. The inanition which accompanies a state of vitamin deficiency is responsible for a nonspecific, reduced sensitivity of the oviduct to the action of estrogen. When this reduced sensitivity is taken into consideration it is found that estrogen exerts a greater than-expected stimulation in thiamine-deficient pullets, probably explainable on the basis of greater availability of estrogen because of failure of inactivating mechanisms.

In riboflavin-deficient pullets the reduced response to estrogen may be accounted for superficially at least, on the basis of inanition alone but may be the algebraic sum of numerous factors such as reduced inactivation of estrogen and decreased functioning of the mechanisms for tissue proliferation. In nicotinic acid-deficient pullets low doses of estrogen can exert a greater-than-expected influence on oviduct growth but high doses are less effective than in inanition controls. A deficiency of folic acid however results in a severe limitation of response to estrogen at all dose levels. The implication is that folic acid is essential to the mechanism of action of estrogen in promoting tissue proliferation in the chick oviduct at the level of the end organ itself.

The relationship of vitamin B₁₂ to stilbestrol action could not be determined on the basis of the data obtained in this experiment. It was found, however, that there is significantly greater response to 3.2 mg. dose of stilbestrol in Sex-linked Cross breed on commercial mash when it is supplemented with vitamin B₁₂, but that supplementation of a synthetic diet with vitamin B₁₂ significantly decreases the oviduct response to this dose of stilbestrol.

*Factors Research Laboratory School of Medicine, and
Leland H. Kaplan
Cleveland, Ohio*

528. MARIKULANDIA, A., MYINT T., and Mc GINNIS, J. Effect of terramycin and vitamin B₁₂ on hatchability. Proc. Soc. Exper. Biol. & Med. 79 242-244, Feb. 1952.

White Leghorn pullets, 8 weeks old, were divided into four groups of approximately 90 birds each. The pullets were kept on range and fed an all-plant protein ration supplemented with 12.5 mcg. of B₁₂ per pound of food. Two groups also received terramycin at a level of 15 ppm.

At 22 weeks of age B₁₂ supplementation was withdrawn from one of the antibiotic fortified groups and from one group receiving the B₁₂ alone. The birds were then housed in individual cages with wire floors and continued on experiment for a period of 10 months.

The vitamin B₁₂ and terramycin supplements alone and in combination had little or no effect on egg production and gain in weight. In the absence of B₁₂, hatchability of fertile eggs was definitely improved by feeding terramycin. There was an indication that terramycin fed in combination with vitamin B₁₂ further improved hatchability.

No great increase in the B₁₂ content of eggs from the terramycin-supplemented lots was observed.

*State College of Washington
Pullman, Wash.*

Mice

529. JAFFÉ, W. G. Reproduction of mice kept on rations low in vitamin B₁₂. Arch. Biochem. 27 464-466, July 1950.

530. JAFFÉ, W. G. Influence of cobalt on reproduction of mice and rats. Science 115 265-267 March 7 1952.

Experiments with 130 litters of mice and 64 litters of rats show that cobalt influences the number of weaned young per litter. Cobalt was administered as CoCl₂ or in vitamin B₁₂. CoCl₂ was given parenterally in drinking water or in food. While the vitamin B₁₂ treated litters had the most weaned young, the animals who received cobalt chloride did almost as well. It is suggested that populations which subsist on diets poor in animal products, and therefore probably poor in vitamin B₁₂, might profit from cobalt supplements.

*Laboratoire National de Nutrition
Carcen, Pennsylvanie*

Rats

531. SURE, B. Vitamin B₁₂ and folic acid for reproduction and lactation. Federation Proc. 9 372, March 1950.

"This work was carried out on the Wistar Strain albino rat. A ration containing 50% low-fat soybean flour furnished 25% proteins in rations which were adequate in all dietary essentials except folic acid and vitamin B₁₂. Ration A was the control without folic acid and vitamin B₁₂; supplementation ration B was supplemented with vitamin B₁₂; ration C was fortified with folic acid, and ration D was supplemented with folic acid and vitamin B₁₂. During a reproduction period of about 4 months the results obtained made it apparent that for optimum reproduction and lactation both folic acid and vitamin B₁₂ are essential and that folic acid plays a significant role in fecundity. Male rats from mothers on ration B weighed after 8 weeks growth, 109 gm. more, and female rats weighed 46 gm. more than rats from mothers on ration A."

*University of Arkansas
Fayetteville, Ark.*

532. LEPKOVSKY, S., BORSON, H. J., BOUTHILLET, R., PENCARZ, R., SINGMAN, D., DIMICK, M. K., and ROBBINS, R. Reproduction in vitamin B₁₂-deficient rats with emphasis upon intrauterine injury. Am. J. Physiol. 165 79-86, April 1951.

Experiments with female rats show that a vitamin B₁₂ deficiency causes them to have progressively severe reproductive abnormalities with successive litters. Third litter young that are born are so debilitated as a result of prenatal injury that they do not survive even when nursed by normal females on stock diet. Vitamin B₁₂ administered parenterally at birth enables many but not all of these third litter rats to survive. Rats that are weaned on vitamin B₁₂-deficient diets undergo a period of high mortality two to three weeks after weaning, generally dying with a severe leukopenia. In general it was found that while lactation was impaired by a vitamin B₁₂ deficiency ability to produce live young was even more impaired, only five live litters resulting from 19 positive matings for a third litter.

*University of California School of Medicine
Berkeley and San Francisco, Calif.*

533. SURE, B. Vitamin B₁₂ in growth, reproduction and lactation. Federation Proc. 10 395, March 1951.

"Results on the influence of the addition of 0.1 µg. daily of crystalline vitamin B₁₂ on growth of the albino rat, during 10 weeks of the post-lactation period, are as follows: As a supplement to a ration containing 25% proteins furnished as low fat soybean flour: 15 males, 20% increased growth; 15 females, 7.2% increased growth. As a supplement to a ration containing 28.2% alpha-soybean protein: 18 animals, 38.5% gain in body weight. As a supplement to 25% proteins in ration furnished as low fat cottonseed flour: 18 animals, 33% increase in body weight. As a supplement to a ration containing 94% as the breakfast food, Cerevim, the addition of 0.1 µg. daily of vitamin B₁₂ for growth, 0.2 µg. for reproduction, and 0.5 µg. for lactation, resulted in marked improvement in reproduction, and lactation efficiency was increased from 37 to 73%. Three generations of animals with excellent growth records were obtained when such vitamin B₁₂-supplemented ration furnished all the B vitamins, proteins and minerals. The addition of 1% APT for

fermentation product of aureomycin to the Carevin ration also produced very favorable effects on reproduction and lactation, and 3 generations of healthy growing animals have been obtained on the latter ration."

University of Arkansas
Fayetteville, Ark.

- 534 SURE, B.: *Vitamin B₁₂ in reproduction and lactation*, J. Am. Dietet. A. 27 554-567 July 1951.

Author's summary: "A diet, the proteins of which were furnished by 50 per cent low-fat soybean flour introducing 25 per cent proteins in the ration and satisfactory in all dietary essentials for good growth of the Wistar strain albino rat, was only partially successful for reproduction and lactation in the first generation. In the second generation, this diet was a marked failure for reproduction and a complete failure for lactation. The supplementation of this basal diet with folic acid resulted in a remarkable improvement in reproduction, but lactation efficiency was only 25 per cent. The addition of vitamin B₁₂ in minute amounts produced a marked increase in lactation efficiency which was 80 per cent. Vitamin B₁₂ also had a favorable effect on reproduction. The addition of both vitamin B₁₂ and folic acid produced the optimum results in both reproduction and lactation of first generation animals. Second and third generation animals were successfully reared on this ration supplemented with B₁₂ only. Fourth generation animals grow well on this diet.

"Excellent results in lactation have been obtained through the second generation with a diet, the proteins of which have been derived from 60 per cent soybean flour supplemented with vitamin B₁₂. This ration unsupplemented with vitamin B₁₂ proved a failure for reproduction and lactation."

University of Arkansas
Fayetteville, Ark.

- 535 SURE, B. *Further studies of vitamin B₁₂ in reproduction and lactation*, Arch. Pediat. 68 540-543, Nov 1951.

The effect of vitamin B₁₂ on reproduction and lactation was studied in rats. The rats were maintained on a basal ration which already contained a good source of vitamin B₁₂ for growth (nonfat milk solids) and to which vitamin supplements (except vitamin B₁₂) were added. The control group received the basal ration plus the vitamin supplements. Another group (3 males and 15 females) were given in addition daily per animal 0.1 µg. of vitamin B₁₂ during growth, 0.2 µg. during reproduction, and 0.5 µg. during lactation periods. In a third group the influence of the addition of APF was studied and in a fourth, some of the basal ration was replaced by dry skim milk. Vitamin B₁₂ significantly reduced the number of stillbirths and increased lactation efficiency by 50.1 per cent in the first generation. In the second generation the further supplementation of the basal ration with folic acid resulted in 100 per cent lactation efficiency. Replacement of some of the basal ration (Carevin) with dry skim milk resulted in an excellent lactation, in the absence of added B₁₂. Replacement of 1 per cent of the basal ration with an APF product produced excellent lactation efficiency and must have replaced the increased needs of vitamin B₁₂

for lactation in the first generation. However there was reduced lactation efficiency in the second generation, even in the presence of folic acid supplementation.

University of Arkansas
Fayetteville, Ark.

- 536 DRYDEN, L. P., HARTMAN A. M., and CARY C. A. *The relation of vitamin B₁₂ deficiency to fertility of the female and birth weight of the young in rats fed purified casein rations*, J. Nutrition 45 877-891, Nov 1951.

A vitamin B₁₂-deficient diet did not effect a greater percentage of nonpregnancies or resorptions in rats than there were in controls on a standard diet, but the litters were smaller and average birth weights lower. B₁₂ supplements improved birth weights, but not litter size.

- 537 MEYER, M. L., THOMPSON H. T., and ELVEHJEM, C. A. *Effect of vitamin B₁₂ on reproduction and lactation in rats receiving pork or beef diets*, J. Nutrition 45 551-565 Dec. 1951.

NUTRITION AND GROWTH

VITAMIN B₁₂; ANIMAL PROTEIN FACTOR

Chicks

- 538 OTT W. H., RICKES E. L., and WOOD T. R.: *Activity of crystalline vitamin B₁₂ for chick growth*, J. Biol. Chem. 174 1047-1048, July 1948 (Letter to Editor)

Crystalline vitamin B₁₂, when added to purified basal diets low in the "animal protein factor" (unidentified chick growth factor) stimulated the growth of chicks as much as or more than dietary supplements of crude sources of this factor. Amounts as small as 6 micrograms per kilogram of diet had this effect. The optimal growth requirements appeared to be less than 30 micrograms per kilogram of diet.

- 539 LILLIE, R. J., DENTON C. A., and BIRD H. R. *Relation of vitamin B₁₂ to the growth factor present in cow manure*, J. Biol. Chem. 176 1477-1478, Dec. 1948 (Letter to Editor)

Crystalline vitamin B₁₂ was found to have activity for chick growth equivalent to that of the unknown growth factor occurring in cow manure and in fish meal and some other feedstuffs of animal origin. The crystalline vitamin B₁₂ was given orally or by injection into the breast muscle. The maximum growth response was the same for crystalline vitamin B₁₂ and for the acid precipitate of water extract of cow manure and essentially the same for the crystalline vitamin and 2-unit liver extract. By calculation, it was found that the acid precipitate contained the equivalent of 5.8 mcg. of vitamin B₁₂. Comparative chick and bacterial assays would be of interest to determine the possible existence of different forms of the vitamin. The injection experiments are particularly significant, since they show that vitamin B₁₂ has a direct effect on the chick, which is not mediated through the intestinal flora.

540. NICHOL, C. A., ROBBLEE, A. R., CRAVENS W. W., and ELVEIHEIM, C. A. *The growth response of chicks to anti-pernicious anemia preparations*, J Biol. Chem. 177 631-639 Feb. 1949

A number of injectable liver extracts and crude liver fractions were tested for growth-stimulating potency in the chick. The stated U.S.P. potency was not related to the growth response. Reticulogen (a liver extract) at 0.01 unit per bird per day caused a half maximal growth response. A vitamin B₁₂ concentrate effectively stimulated chick growth at a level of 1.5 mcg. of vitamin B₁₂ per 100 Gm. of ration. However the concentrate used did not exclude the presence of other factors such as those that occur in reticulogen. Other liver extracts (15 U.S.P. units) active in the remission of pernicious anemia, were inactive for the growth of the chick. Two crude liver fractions which are discarded in the commercial preparation of anti-pernicious anemia extracts were very active in promoting chick growth.

541. OTT W. H.: *Further studies of the activity of crystalline vitamin B₁₂ for chick growth* (Paper presented at American Chemical Society meeting Symposium on vitamin B₁₂ and related factors) Atlantic City Sept. 19-23 1949

Chicks from hens fed only an all-vegetable protein ration were given a diet consisting of 70 per cent soybean meal plus purified nutrients (vitamins, minerals, amino acids) known to be needed by the chick, and lacking only in nutrients having vitamin B₁₂ activity. Crystalline vitamin B₁₂ was then added at various levels (the control group receiving none) and the growth responses determined. From the results obtained it was estimated that the minimum vitamin B₁₂ requirement for maximum early growth of chicks was 2.7 mcg. per 100 Gm. of ration. A probable maintenance requirement of not less than 0.01 mcg. per 100 Gm. was indicated. The vitamin B₁₂ requirements apparently were increased when dried whey alfalfa meal, and certain other natural products were included in the diet.

542. MILLER, D. C., and GROSCHKE, A. C.: *The occurrence of "animal protein factor" in horse manure as measured by chick growth responses*, Michigan Agric. Exper. Sta. Quart. Bull. 52 279-287 Feb. 1950.

Incubated horse manure was found to be a potent source of AFP while fresh horse manure contained no activity (Same as previously reported for chick feces.) This shows that intestinal synthesis of AFP does not occur in the horse. "This observation pointed out the importance of certain aerobic bacteria in the synthesis of AFP after defecation and that synthesis of AFP did not occur in the intestine of chickens."

A charcoal adsorbate of vitamin B₁₂ was shown to have AFP activity

543. COMBS G. F., CARLSON C. W., MILLER, R. F., PEELER, H. T., NORRIS, L. C., and HEUSER, G. F.: *Studies of unidentified chick growth factors*, J Biol. Chem. 182 727-737 Feb. 1950.

Studies of growth-stimulating factors in counter current distribution fractions obtained from refined liver paste dialysate indicate the existence of four unidentified substances which promote rapid early growth in chicks. An improved microbiologic assay with *Lactobacillus leichmannii* gave evidence that two of these factors are different forms of vitamin B₁₂. The other substances do not appear to be identical with vitamin B₁₂ or with any of the known vitamins. Significant growth responses have been obtained by feeding fractions containing either of these unknown factors at levels which supplied approximately 0.03 to 0.04 mcg. of vitamin B₁₂ per 100 Gm. of diet. This level of vitamin B₁₂ is too low to have any material growth-promoting effect on chicks. A combination of fractions containing these two unknown factors appeared to indicate a mutual supplementary effect.

Lactobacillus leichmannii responds in the presence of adequate vitamin B₁₂ to an unknown factor obtained from countercurrent distribution fractions of liver paste, which also contains one of the unknown chick growth factors. *Lactobacillus casei* also responds to these fractions. Whether or not the chick and microbiologic factors are identical has not been determined.

Cornell University
Ithaca, N. Y.

544. GASSNER, F. X., PATTON, A. R., WILGUS, H. S., and GHARKEY, L. W. *Failure of cockerel comb and testis development on sesame meal and its prevention by vitamin B₁₂*, Proc. Soc. Exper. Biol. & Med. 75 630-633, Dec. 1950.

545. COUCH, J. R., OLCESE, O., SANDERS, B. G., and HALICK, J. V.: *Vitamin B₁₂, AFP concentrates dried whey fish solubles and liver fraction "L" in the nutrition of the mature fowl*, J Nutrition 42 473-485, Nov. 1950.

546. MENGE, R., MORENG, R. E., and COMBS, G. F.: *Effect of yolk removal in day-old chicks on early nutritional requirements*, Proc. Soc. Exper. Biol. & Med. 76 46-49 Jan. 1951.

Rats

547. EMERSON, G. A., WURTZ, E., and ZANETTI, M. E.: *Vitamin B₁₂—a growth factor for young rats*, Federation Proc. 8 381-382, March 1949

Size and birth weights of the litters cast by rats that received 5 mcg. vitamin B₁₂ daily from the time of impregnation were the same as those of litters cast by untreated rats. The weaning weights (at 8 days) of the offspring of the mothers receiving vitamin B₁₂, however averaged 50 per cent more than those of the offspring of the untreated mothers. At weaning, each group was separated into two groups, one of which received 0.5 mcg vitamin B₁₂ daily for 90 days while the other received no supplement. The weight increments of the males from the different groups were as follows

Males from control mothers, young weaned	151 Gm.
Males from control mothers, young weaned	237 Gm.
Males from treated mothers, young weaned	279 Gm.
Males from treated mothers, young weaned	283 Gm.

The feeding of vitamin B₁₂ to the young of control mothers resulted in body weights 100 Gm. in excess of those of the unsupplemented young of these mothers. Since the undersized young of the treated mothers grow for two months at a rate approximating that of the dosed young from the control mothers, it would appear that vitamin B₁₂ is stored during the suckling period. The red and white cells of the individuals of all groups fell within the normal range.

548. HARTMAN A. M., DRYDEN L. P., and CARY C. A. *Bacterial synthesis in the rat of an unidentified growth-promoting factor* Federation Proc. 8 205, March 1949

Some rats on a diet deficient in an unidentified growth-promoting vitamin (X) supplemented with unusually high levels of the ten B vitamins plus methionine and vitamins C, E, and K, gained weight nearly twice as fast as others on the same diet. The slow-growing rats responded to a single small dose of feces from those that grow rapidly. This suggests that X may be synthesized by microorganisms which may be induced to thrive in the rat. This synthesis was made possible by the extraordinarily high level of riboflavin. Findings reported by Stokstad, by Ott, and by Bird (Abstr. 84 538, 539) are cited which suggest that the animal protein factor antipernicious anemia factor X, and vitamin B₁₂ are either very closely related or identical. X occurs in different combinations in different feeds; all are active in the normal rat.

549. HARTMAN A. M., DRYDEN L. P., and CARY C. A. *A role of vitamin B₁₂ in the normal mammal*, Arch. Biochem. 33 165-168, Aug. 1949

The authors have compared crystalline vitamin B₁₂ with APA 15-unit liver extract fed to weanling young rats as supplements to X-deficient basal rations adequate in other nutrients and containing 25, 45, or 65 per cent protein. The test young were reared by mothers that were on a X-deficient ration during the nursing period. The 14-day growth on the basal ration containing an X deficient casein decreased from a weight gain of 36 Gm. to 22 Gm. and 4 Gm., respectively with these increases in the percentage of protein. Supplementation with vitamin B₁₂ increased the rate of growth on all levels of protein, and maximally effective doses of vitamin B₁₂ produced practically the same effect as doses of the liver extract which the authors previously had found effective. The results show that with all levels of protein tested a deficiency of vitamin B₁₂ has a deleterious effect on growth. Apparently vitamin B₁₂ plays a fundamental role affecting the capacity of the normal mammal to utilize protein.

550. PICCIONI, M., RABBI, A., and MORUZZI, G. *Animal protein factor for the rat present in crude casein and its relationship with vitamin B₁₂*, Science 113 179-181, Feb. 16, 1951.

Crude casein contains a factor (or factors) which is not B₁₂, that is indispensable for the normal growth and reproduction of rats.

551. CUTHBERTSON W F J., and THORNTON D M. *Effect of parenteral nutrition on the growth of the rat to vitamin B₁₂*, Brit. M. J 5 xii, 1951.

Swine

552. HOGAN A. G., and ANDERSON G. C.: *Vitamin B₁₂ in swine nutrition*, Federation Proc. 8 885-886, March 1949

Six pigs were taken from their mothers at the age of two days and reared on a synthetic milk diet including vitamin-free casein, sucrose, corn starch, lard, mineral salts, and generous supplies of vitamins. Three of them were given intramuscular injections of crystalline vitamin B₁₂ at three-day intervals until they were 36 days old. The total dosage of B₁₂ was 50 mcg. in one pig, 100 mcg. in the second, and 200 mcg. in the third. In the following four-day period the pigs that received vitamin B₁₂ made an average gain of 26.8 pounds and the others gained 15.9 pounds. In the following six-week period one of the pigs that did not receive vitamin B₁₂ died unexpectedly one did not gain consistently and the third began to decline but after injections of vitamin B₁₂ totaling 15 mcg. began to gain at a moderate rate. The pigs that received vitamin B₁₂ grew at a uniform rate, with an average gain of 58.8 pounds, which is exceptional for that age.

553. NEUBMANN A. L., JAMES, M. F., KRIDER, J. L., and JOHNSON, B. C.: *Essentiality of vitamin B₁₂ for the baby pig, with preliminary quantitative data*, Federation Proc. 8 891 March 1949

The addition of a vitamin B₁₂ concentrate to isolated soybean protein synthetic milk improved the growth and physiologic well-being of baby pigs. The maximum growth response was obtained with 42 mcg. per Kg. of dry matter in the diet, and hemopoiesis was improved by this diet. The vitamin B₁₂-deficient pigs were irritable, sensitive to touch, and sluggish in their movements; a number of them could not stand because of weakness in their rear legs. The pigs that received vitamin B₁₂ at sufficient levels had none of these symptoms and grew more rapidly. A manure factor concentrate gave good growth but did not protect against the gross symptoms observed in the vitamin B₁₂-deficient pigs.

554. JOHNSON B. C., and NEUBMANN A. L. *Crystalline vitamin B₁₂ compared to antipernicious anemia liver extract for pig growth*, J Biol. Chem. 178 1001-1002, April 1949

The responses of baby pigs to 2 mcg. of vitamin B₁₂ and to reticogen at 0.1 ml. per day were equal.

555. LUECKE, R. W., McMILLEN W N., THORP F., JR., and BONIECE, J R. *The effect of vitamin B₁₂ concentrate on the growth of weanling pigs fed corn-soybean diets*, Science 110 189-190 Aug. 5, 1949

Weanling pigs that were fed a corn and soybean diet supplemented with 0.5 per cent vitamin B₁₂ concentrate gained weight at a significantly greater rate than pigs fed the same basal ration without the supplement. By the end of a seven week trial period they averaged 17 lbs. heavier than their controls. Only 2.65 lbs. of supplemented feed was required to produce a 1 lb. gain in body weight compared with 2.94 lbs. of unsupplemented feed, which indicated that the concentrate effected more efficient utilization of the feed. It seemed probable that vitamin B₁₂

per se was responsible for the growth promoting activity of the concentrate, although it cannot be definitely stated that this was the case since the latter undoubtedly contained impurities.

555. ANDERSON G. C., and HOGAN A. G.: *Requirement of the pig for vitamin B₁₂*, J Nutrition 40 243-253, Feb. 1950.

The tentative estimate of the quantitative requirement of the pig for vitamin B₁₂ when administered orally is 0.26 mcg. daily per Kg. of live weight, or not over 1.5 mcg. per 100 Gm. of food.

Two pigs supplied with fortified cows milk grew more rapidly than did those that received vitamin B₁₂. This suggests that an unrecognized vitamin is essential for the optimum nutritional state.

557. NEUMANN A. L., and JOHNSON B. C.: *Crystalline vitamin B₁₂ in the nutrition of the baby pig*, J Nutrition 40 403-414, March 1950.

Crystalline vitamin B₁₂ has been shown to be required by the baby pig. It is the growth factor which is supplied by the anti-pernicious anemia liver extract. The relation between the deficiency produced in baby pigs and pernicious anemia in man is discussed.

558. NESHEIM, R. O., KRIDER, J. L., and JOHNSON B. C.: *The quantitative crystalline vitamin B₁₂ requirement of the baby pig*, Arch. Biochem. 27 240-242, June 1950.

Requirement of vitamin B₁₂ given by injection (0.6 mcg./Kg. body weight) was found to be one half that by oral administration (20 mcg./Kg. of dry matter consumed).

559. BURNSIDE, J. E., CUNHA, T. J., EDWARDS H. M., MEADOWS G. B., LAMAR, G. A., PEARSON A. M., and GLASSCOCK, R. S.: *Response of the pig to AFP B₁₂ and B₁₂ Proc. Soc. Exper. Biol. & Med.* 74 173-174, May 1950.

A mixture of B₁₂ and B₁₂ was of no benefit for growth when administered by injection or orally to the pig during a 40-day period under the conditions of this experiment. AFP prevented a diarrhoea which occurred periodically with the other pigs on experiment. AFP fed pigs exhibited a smoother hair coat and more bloom. Further evidence was obtained to show that AFP contains a factor(s) other than B₁₂ or B₁₂.

560. BOOTH, A. N., ELVEHJEM, C. A., and HART E. B.: *The importance of bile in the nutrition of the guinea pig*, J Nutrition 37 263-274 Feb. 1949.

A purified basal ration containing all the known nutrients, except vitamin B₁₂, produced a growth rate of only 1.8 Gm. per day for six weeks in guinea pigs compared to a normal rate of 6.9 Gm. per day when a commercial ration composed of natural feeds was fed. A nearly normal average growth rate of 6.5 Gm. per day was obtained when the purified basal ration was supplemented with 25 per cent alfalfa leaf meal in place of an equal amount of sucrose. Growth was equally good when

the basal ration was supplemented with 30 per cent dried beet pulp. Powdered gum arabic fed at a level of 15 per cent produced an average growth rate of 5.1 Gm. per day. Nearly normal growth rates were obtained for periods as long as twelve weeks, on the 15 per cent gum arabic ration by the addition of the ash from alfalfa meal equivalent to 25 per cent. When gum arabic was fed, the level of protein in the synthetic diet could be reduced.

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Madison, Wis.

Other Animals

561. SCHEID H. E., MCBRIDE, B. H., and SCHWEIGERT B. S.: *The vitamin B₁₂ requirement of the Syrian hamster*, Proc. Soc. Exper. Biol. & Med. 75 236-239 Oct. 1950.

Syrian hamsters were kept on either a corn-soybean oil meal ration with or without iodinated casein, or a casein basal ration with or without iodinated casein for a two-week depletion period. Each group was then divided into three subgroups, which received either the basal ration, the basal ration plus 50 mcg. of vitamin B₁₂ per Kg. of ration, or the basal ration plus 2 per cent whole liver substance (which may provide additional unknown factors as well as vitamin B₁₂). These supplements produced a small gain in weight only in the animals on the corn-soybean and iodinated casein diet in the other groups there was either no difference in growth or slightly less growth in the animals receiving the supplements. A vitamin B₁₂ requirement of the hamster therefore, was not demonstrated.

In parallel experiments with rats on the corn-soybean oil meal ration plus iodinated casein, a vitamin B₁₂ requirement was demonstrated. A reduction in food efficiency attributable to vitamin B₁₂ deficiency was observed in these animals.

American West Institute Foundation, and
The University of Chicago
Chicago, Ill.

562. SCHWEIGERT B. S.: *The animal protein factor*, Nutrition Rev 7 225-227 Aug. 1949.

The author provides a list of the animal protein factors that have been studied most extensively including their sources, and discusses their occurrence, the nature of the nutritional deficiencies produced in various animals, and interpretations of the results obtained. Ample evidence is available demonstrating that unknown factors are required by the chick; vitamin B₁₂ is capable of replacing the active fractions from cow manure, liver extract, and fish solubles, either with a soybean oil meal-corn or casein basal ration. It is pointed out that the various animal protein factors may be attributable to one chemical entity or its conjugates, as in the case of folic acid. The factors replaceable by folic acid are tabulated. It is suggested that a similar tabulation for factors with activity attributable to vitamin B₁₂ may be made in the near future.

563. ZUCKER, T. F., and ZUCKER, L. M.: *"Animal protein factor" and vitamin B₁₂ in the nutrition of animals*, Vitamins & Hormones 8 154, 195

564. ASCHKENASY A. *La vitamine B₁₂ et le facteur des protéines animales (Vitamin B₁₂ and the animal protein factor)* Ann. Nutr. Aliment. 4 141 167 1950

COBALT REQUIREMENT OF SHEEP

563. BECKER, D. E., SMITH, S. E., and LOOSLI, J. K. *Vitamin B₁₂ and cobalt deficiency in sheep*, Science 110 71 72, July 15, 1949

Cobalt-deficient lambs have been found to respond quickly by improved appetite, weight gain, and increased blood hemoglobin when fed, but not when injected, with 1 mg. of cobalt daily. This suggested, assuming vitamin B₁₂ to be a necessary metabolite for sheep, that cobalt was synthesized into vitamin B₁₂ by the rumen flora when it was administered orally but that injected cobalt was incapable of this transformation. A study was therefore made of the effect of oral or intramuscularly injected vitamin B₁₂ in cobalt-deficient lambs. The dosage of the material was arbitrary one animal receiving as little as 2 micrograms and another as much as 120 micrograms weekly. The larger amounts were administered orally. Injections were given twice weekly. Regardless of the amount of the dosage, neither route of administration produced a response over periods of five to six weeks, as judged by hemoglobin levels and weight gains. The lack of response to vitamin B₁₂ in these tests lends no support to the theory that this factor is an important intermediary in cobalt metabolism in lambs.

566. MARSTON H. R., and LEE, H. J. *Primary site of the action of cobalt in ruminants* Nature 164: 529-530, Sept. 24, 1949 (Letter to Editor)

More than 10 years ago the authors discovered that cobalt, given orally was dramatically effective in coast disease, a fatal malady affecting sheep confined to cobalt deficient pastures. Their later experiments have shown unequivocally that for the cobalt to be effective it must be ingested, and that cobalt introduced parenterally is of no benefit. They conclude that cobalt exerts its influence on the ruminant primarily either in the lumen of the alimentary canal or when passing through its wall, the site of its activity being apparently above the level of the duodenum since cobalt given intravenously appears in the intestinal tract below this level. A probable important function of cobalt in the ruminant may concern the activity of the symbiotic flora within the paunch. Sheep and cattle are apparently the only animals affected by cobalt-deficient grazing land rabbits thrive on it and horses do exceptionally well. This implies that the ruminant is unique in its higher demand for the element.

The authors refer to evidence that neither the anemia nor other symptoms of extreme cobalt deficiency respond to Minot's anti-pernicious anemia factor introduced parenterally either as concentrated liver extract or as crystalline vitamin B₁₂, in quantities that would rapidly correct the dyscrasia of human pernicious anemia. It would now appear that the physiologic role of cobalt is not restricted to the part it takes in the maturation of erythrocytes in the bone marrow.

*Consult for Editorial and Production Research
Schubert, A. and others*

567. ABELSON P. H., and DARBY H. H. *The synthesis of vitamin B₁₂ in the digestive system of the sheep*, Science 110 566, Nov 25, 1949

About one half the amount of cobalt fed to sheep was excreted in organically bound form in the feces.

Biological assay of the butanol extract indicated the presence of large amounts of vitamin B₁₂ thus sheep feces appear to be an important source of this vitamin.

*Consult Institution of Washington
Washington, D. C.*

568. HARPER, A. E., RICHARD R. M., and COLLINS, R. A. *The influence of dietary cobalt upon the vitamin B₁₂ content of ewe's milk*, Arch. Biochem. & Biophysics 31 323-329 April 1951.

The supplementation of cobalt or trace-minerals (containing cobalt) to the ration of ewes produced a highly significant increase in the vitamin B₁₂ content of the ewe's milk. It is probable that this observation is the result of increased vitamin B₁₂ synthesis by the rumen microorganisms.

569. SMITH, S. E., KOCH, B. A., and TURK, E. L. *The response of cobalt-deficient lambs to liver extract and vitamin B₁₂*, J. Nutrition 44: 455-464, July 1951.

The curative activity of an anti-pernicious anemia liver extract when injected into cobalt-deficient lambs has been confirmed. There was a high correlation between the vitamin B₁₂ activity of these liver extract fractions and the response of the deficient animals. The presently available data show that vitamin B₁₂ is an important intermediary in cobalt metabolism in sheep.

FOLIC ACID; OTHER VITAMINS

570. NICHOL, C. A., DIETRICH, L. S., ELVEHJEM, C. A., and HART E. B. *Observations on folic acid deficiency in the chick in the presence of vitamin B₁₂*, J. Nutrition 39 237-238, Nov 1949

Folic acid deficiency in chicks was accentuated by oral or parenteral administration of vitamin B₁₂. Folic acid treatment completely counteracted the symptoms within 24 hours. B₁₂ increased the growth rate but not the feathering in folic acid-deficient chicks.

571. DIETRICH, L. S., NICHOL, C. A., MONSON, W. J., and ELVEHJEM, C. A. *Observations on the interrelationship of vitamin B₁₂, folic acid and vitamin C in the chick*, J. Biol. Chem. 181: 915-920, Dec. 1949

Vitamin C and B₁₂ stimulate growth of chicks fed a folic acid-deficient diet.

Vitamins C and B₁₂ stimulate synthesis of folic acid folic acid stimulates the synthesis of B₁₂.

572. EVANS R. J., GROSCHKE, A. C., and BUTTS, H. A. *Effect of vitamin B₁₂ on peroxisomal acid metabolism in the chick*, Arch. Biochem. & Biophysics 31 454-456, May 1951.

Chicks that were more deficient in vitamin B₁₂ had higher liver pantothenic acid contents.

"Our tentative explanation of the data obtained in that vitamin B₁₂ aids in the transfer of pantothenic acid from the liver for use elsewhere in the body and that in the absence of sufficient vitamin B₁₂, pantothenic acid accumulates in the liver."

573. YACOWITZ, H., NORRIS, L. C., and HEUSER, G. F.: Evidence for an interrelationship between vitamin B₁₂ and pantothenic acid. *J. Biol. Chem.* 192 141-146, Sept. 1951.

Vitamin B₁₂ was found to spare the pantothenic acid requirement for growth, survival, and the prevention of dermatitis in chicks with normal reserves of vitamin B₁₂.

In turn, pantothenic acid showed a sparing effect on the vitamin B₁₂ requirement for growth of normal chicks.

References are given for interrelationships between vitamin B₁₂ and amino acids in general, methionine, glycine, choline, betaine, folic acid and ascorbic acid.

Most pantothenic acid in tissues is present in coenzyme A.

574. HOVE, E. L., and HARDIN, J. O. Relation of vitamin B₁₂ to vitamin E in nutrition of young rats. *Federation Proc.* 9 362, March 1950.

"Previous work has shown that supplements of alpha tocopherol given to young rats on a low casein, vitamin E-free diet increased growth and protein utilization, gave marked protection against CCl₄, and prevented a fatal lung hemorrhage-liver necrosis syndrome which occurred after about 3 months. The diets used in this work contained no added source of vitamin B₁₂, folic acid or biotin. (Hove, E. L., D. H. Copeland and W. B. Salmon. *J. Nutrition* 39: 397 1949.) When boiled egg white or fibrin replaced casein as the protein source at 10% supplements of 1 mg. alpha tocopherol daily did not increase growth or give protection against CCl₄. However on diets with defatted whole wheat or soybean meal as the protein source, vitamin E increased growth and protein utilization. When vitamin B₁₂ concentrate was added at a level equivalent to 80 µg./kg. to diets containing soybean meal, casein or whole wheat, the protein efficiency ratios increased sharply and vitamin E supplements were without apparent benefit. Folic acid or biotin did not influence the results. Supplements of vitamin B₁₂ offered some protection against CCl₄, but under the conditions used could not replace vitamin E. The combination was more effective than either alone. Against the fatal lung hemorrhage-liver necrosis syndrome vitamin B₁₂ gave little protection although in some experiments the time of onset was delayed."

*Abstracts Polytechnic Institute
Brooklyn, N.Y.*

575. DINNING, J. S., KEITH, C. K., PARSONS, J. T., and DAY, P. L. The influence of pteroylglutamic acid and vitamin B₁₂ on the metabolism of pyridine-fed rats. *J. Nutrition* 42 81-89, Sept. 1950.

576. ERSHOFF, B. H., and McWILLIAMS, H. B.: Effects of B vitamins and liver on growth of immature rats maintained at low temperatures. *Proc. Soc. Exper. Biol. & Med.* 75 226-229 Oct. 1950.

Immature rats kept in low temperatures were fed a purified ration supplemented, in some instances, with dehydrated whole liver, water insoluble liver residue, or a water-soluble liver extract. Body weight and gonadal weight increased less rapidly in the control rats kept in the cold room than in those under room temperature conditions. The supplements resulted in a marked increase in both body and gonadal weight. A supplement of the known B vitamins was similarly effective. It is suggested that the protective effects of the various supplements were due, at least in part, to their vitamin B₁₂ content.

*Emory V. Thompson Laboratories
Los Angeles, Calif.*

577. VERLY, W. G., WILSON, J. E., KINNEY, J. M., and RACHELE, J. R. Action of vitamin B₁₂ and folic acid on labile methyl synthesis in the rat. *Federation Proc.* 10 264 March 1951.

"It has been shown that the carbon of methanol can be incorporated into the methyl group of choline in the rat (du Vigneaud and Verly *J. Am. Chem. Soc.* 72 1049 1950). Experiments with methanol labeled with deuterium and C¹⁴ indicated that methanol was used, presumably through oxidation and subsequent reduction, in the synthesis of a methyl group in the formation of a labile methyl compound (du Vigneaud, Verly, Wilson, Rachelle, Rosaler and Kinney *J. Am. Chem. Soc.* In press). This newly formed methyl group then enters transmethylation reactions. Experiments have now been undertaken to see whether vitamin B₁₂ or folic acid had any effect on the utilization of methanol. C¹⁴ Deuterio-methanol was injected into rats subcutaneously. Tri-methylamine derived from choline isolated from the carcasses was analyzed for C¹⁴ and deuterium. No significant difference in the utilization of methanol for labile methyl synthesis was found between young, B₁₂-deficient rats and their litter mates prepared in the same way but injected with B₁₂ prior to administration of methanol. Folic acid administration caused a two-to-threefold increase in the utilization of methanol in labile methyl synthesis in the folic acid-deficient rat. These results on B₁₂ and folic acid supplement those obtained by Sakami and Welch (*J. Biol. Chem.* 187: 579 1950) on labile methyl synthesis from formalin in liver slices."

*Cornell University Medical College
New York, N.Y.*

578. HARTMAN, A. M., DRYDEN, L. P., and CARY, C. A.: The effect of riboflavin on the bacterial synthesis of vitamin B₁₂-active material in the rat. *Arch. Biochem. & Biophysics* 34 324-338, Dec. 1951.

The results of this study indicated bacterial synthesis of B₁₂-active material in the intestinal tract which was shown to be due to high levels of dietary riboflavin.

579. ENGEL, R. W., and ALEXANDER, H. D.: Prevention of nutritional edema with vitamin B₁₂ and folic acid. *Federation Proc.* 11 441-442, March 1952.

"A choline-low diet containing 7% of protein produced a high incidence of generalized nutritional edema and severe anemia in weanling rats in an average period of 90 days. The edema and anemia were prevented by supplementing the diet with 0.2% of choline chloride (Engel and Alexander *Federation Proc.* 10 882, 1951). The edema was completely prevented and hemoglobin levels of 11 gm./100 ml. of blood were maintained for 300 days when the choline-low diet was supplemented with 50 µg. of vitamin B₁₂ and 2 mg. of folic acid/kg. of diet. Rats receiving this supplement made average body weight gains of 120 gm. during the experiment, contrasted with average body weight gains of only 20 gm. in rats protected from edema with a supplement of choline. A supplement of folic acid alone did not prevent the edema or the anemia although it appeared to prolong survival. Rats receiving this supplement failed to grow and developed edema in 120 days, at which time the blood hemoglobin levels had decreased to between 2.2 and 6.1 gm/100 ml. of blood."

*Johnson Polytechnic Institute
Akron, Ohio*

580. TOVE, S. B., and ELVEHJEM, C. A. *Relation of factor in a methanol extract of liver and pteroylglutamic acid in milk*, *Federation Proc.* 8 399 March 1949

In the experiments described, it was found that the pteroylglutamic acid deficiency syndrome in milk can be relieved or prevented by administration of a methanol extract of fresh liver. The pteroylglutamic acid content of blood, urine and feces of the animals was greater than could be accounted for by the pteroylglutamic acid in the extract. Vitamin B₁₂ preparations or commercial liver extracts used in pernicious anemia did not produce the response obtained with the methanol extract. This indicates that the methanol extract factor which is different from vitamin B₁₂ has a sparing action on pteroylglutamic acid.

- 581 TOVE, S. B., LALOR, R. J., and ELVEHJEM, C. A. *Properties of the methanol soluble factor required by the milk*, *Proc. Soc. Exper. Biol. & Med.* 75 71 74, Oct. 1950

The methanol extract factor required by the milk is soluble in 90 per cent phenol and insoluble in acetone, n-butanol and 5% saturated ammonium sulfate. It is contained in fish solubles, but not in dried distillers solubles, dried whey, pork spleen, or hog intestinal mucosa. It will be noted that there is a good correlation between the content of this factor and of vitamin B₁₂ in the various products. The solubilities of this factor and of vitamin B₁₂ are similar. The classic symptoms of pernicious anemia are seen in milk deficient in the methanol extract factor. In the chick and the rat, vitamin B₁₂ has been shown to be effective in preventing fatty degeneration of the liver and kidneys—a similar condition in the milk is cured by the methanol extract. Since the methanol extract is active at a daily intake of less than 1 cc., which contains only 2 mcg. of crystalline vitamin B₁₂, while the crystalline vitamin B₁₂ is effective only in doses of 10 to 20 mcg., the methanol extract factor must have an effect in addition to the actual amount of B₁₂ present. It would seem

that the methanol extract factor may be another form of vitamin B₁₂.

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Madison, Wis.*

582. JOHNSON R. C., NEUMANN A. L., NESHEIM, R. O., JAMES M. F., KRIDER, J. L., DANA, A. S., and THIERSCH, J. B.: *The interrelationship of vitamin B₁₂ and folic acid in the baby pig* *J. Lab. & Clin. Med.* 36 537-546 Oct. 1950.

Authors summary "Baby pigs fed a vitamin B₁₂ deficient diet for three weeks were co-fed 8 Gm. of x methyl folic acid per kilogram of dry matter of the diet for the next two weeks, which resulted in a marked decrease in growth rate and in the death of five out of twelve pigs.

"At the end of the three-week depletion period, the bone marrow showed a rise in nucleated red cells. Following the two-week feeding of the folic acid antagonist the bone marrow became depleted, especially in erythroid elements, and numerous basophilic normoblasts and erythroblasts appeared.

"The blood and bone marrow symptoms of this combined vitamin B₁₂-folic acid deficiency were cured by either crystalline vitamin B₁₂ or by folic acid therapy.

"Vitamin B₁₂ therapy resulted in optimum growth, while folic acid treatment gave only temporary and sub-optimal growth stimulation.

"In a second experiment baby pigs were fed a lower protein (20 per cent as compared with 50 per cent in the previous experiment) vitamin B₁₂-folic acid low diet plus Sulfathiazidine in an attempt to produce the double deficiency without the use of a folic acid antagonist.

"In this experiment a marked reticulocyte response occurred on vitamin B₁₂ therapy followed by a second marked response to folic acid administration.

"The vitamin B₁₂-deficient pigs were found to have enlarged thyroids, kidneys, livers, and tongues.

"From these two experiments it appears that the pig requires both vitamin B₁₂ and folic acid and that both are involved in hematopoiesis. In addition, vitamin B₁₂ is required for normal growth."

*University of Illinois
Urbana, Ill.
Shaw-Neering Institute for Cancer Research
New York, N. Y.*

- 583 REID M. E. *Development of a synthetic diet for young guinea pigs and its use in the production of specific B-vitamin deficiencies* *Federation Proc.* 12 473, March 1953.

"A synthetic diet containing vitamin-free casein, sucrose, corn starch, sorbitol, cellophane, corn oil, minerals and vitamins has been developed for guinea pigs, starting them at 2-4 days of age and continuing up to 8 weeks of age. With respect to general health, rate of growth, appearance and size of the internal organs, and the blood picture, this diet produced animals similar to a) those reared on the same diet supplemented with kale, b) those on a commercial rabbit chow diet with ascorbic acid

added, and c) those on diet b but supplemented with kale. The omission of individual B vitamins from the diet in different experiments, started with guinea pigs 2-4 days old, has led to the production of clear-cut deficiencies of thiamine, niacin, choline, pantothenic acid, pyridoxine, folic acid, and riboflavin. Present results suggest that vitamin B₁₂ and inositol are not essential for survival but may be essential for maximum growth. It appears that biotin in the diet may not be needed but additional studies are necessary. The quantitative requirement of the different B vitamins is being determined."

Personal Narrative of Health
Bryn Mawr, Pa.

- 584 FATTERPAKER, P., MARFATIA, U., and SREENIVASAN, A.: *Influence of folic acid and vitamin B₁₂ on formation of creatine in vitro and in vivo* Nature 167 1067-1068, June 30, 1951

In Swiss mice, deficiency of either folic acid or vitamin B₁₂ causes a diminution in creatine formation and excretion together their effects are additive. There is reason to believe that creatine formation is primarily influenced by folic acid and that vitamin B₁₂ exerts its effect by promoting better utilization of folic acid at low levels. It is possible that in the absence of folic acid there is a drain on the methyl economy due to methionine and choline, giving rise to formate that formate utilization is favored by folic acid is now known. The favorable effect of vitamin B₁₂ could not be observed in attempts at in vitro activation of the creatine-forming system by additions of vitamin B₁₂ solutions.

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Mumbai, Bombay 20 India

- 585 OLESON, J. J.: *Studies on pteroylglutamic acid inhibitors* Trans. N. Y. Acad. Sc. 12 118-121, Feb. 1950.

Biological studies on a number of pga derivatives are reported. Certain interrelationships between pga derivatives and dietary protein and vitamin B₁₂ have been illustrated.

PROTEINS, FATS CARBOHYDRATES

586. HARTMAN, A. B., DRYDEN, L. P., and CARY, C. A.: *The role and sources of vitamin B₁₂* J. Am. Dietet. A. 25 929-933, Nov. 1949.

The authors fed various diets deficient in vitamin B₁₂ to weanling rats. It was found that incredibly small doses of B₁₂ (as low as 0.01 mcg. daily) suffice to produce significant increases in the rate of growth of rats. The feeding of B₁₂-deficient rations with high levels of protein was deleterious—sometimes even fatal. It is, therefore, concluded that vitamin B₁₂ plays an important role in determining the capacity of the normal mammal to utilize protein. Furthermore, vitamin B₁₂ plays an important role in the growth and development—and probably in the reproduction and lactation—of the normal mammal. Various foods and feeds tested for B₁₂ activity by feeding them in, or as a supplement to, basal rations are shown in the accompanying table.

FOODS AND FEEDS

WITHOUT B₁₂ ACTIVITY

White flour
Enriched white flour
Whole wheat flour
Wheat bran
Yeast (baker's or brewer's)
Caramel (yellow)
Soybean oil meal
Lime oil meal
Corn oil
Dietrich's dried milk
Cottonseed flour (hydrolyzed, defatted)
Cane molasses
Tomatoes
Egg white (heat-coagulated)
Butterfat

WITH B₁₂ ACTIVITY

Milk
Skim milk (dried or dried)
Cheese (cottage, Swiss, Cheddar)
Cocoa, whole
Liver extracts
Beef muscle
Pork muscle
Egg yolk
Lard
Alfalfa or alfalfa hay
Timothy hay
Kentucky blue grass
Blue polyploid concentrate

The authors have calculated that 1 quart of milk, when fed to a normal rat, has a potency equivalent of 10 or 12 mcg. of crystalline B₁₂.

U. S. Department of Agriculture
Beltsville, Md.

- 587 CUNHA, T. J., BURNSIDE, J. E., EDWARDS, H. M., MEADOWS, G. B., BENSON, R. H., PEARSON, A. M., and GLASSCOCK, R. S.: *Effect of animal protein factor on lowering protein needs of the pig*, Arch. Biochem. 25 455-457 Feb. 1950.

Efficient utilization of protein by pigs was obtained only when the diet was supplemented by AFP.

The accepted values for the protein requirements of swine may need to be reevaluated by using adequate amounts of vitamin B₁₂, plus other factors present in the AFP supplement in the ration.

588. KRZYWICKI, H., and DACOSTA, E.: *Effect of high protein unsupplemented and the same supplemented by B₁₂ after dietary restriction on the spontaneous activity of the rat*, Am. J. Physiol. 171 741, Dec. 1952 (in Soc. Proc.).

"Spontaneous activity body weight and food intake were studied on 96 rats during either diet 1 (caloric restriction) diet 2 (2% protein) diet 3 (4.5% protein and 10% salt) and during rehabilitation on diet 4 (60% protein ad libitum) diet 5 (diet 4 limited to 11 gms/day) and diet 6 (diet 4 plus B₁₂). During rehabilitation after diet 1 the activity of the group on diet 5 rose during the first 9 weeks to 2355 revolutions but at the end of 13 weeks all groups were at about the same level (627 revolutions). The activity of the groups on diet 6 and 4 after diet 2 rose rapidly together for the first 10 weeks. After 13 weeks of rehabilitation the activity of the groups on diet 6 was 3373 revolutions compared to 4244 for the diet 4 group and 1371 for the diet 5 group. During rehabilitation after diet 3 the activity of the group on diet 5 was greater than that of the other rehabilitants but all were below the controls. Thus, the previous dietary history determines the activity response during rehabilitation on a high protein diet."

U. S. Army Medical Research Laboratory
Chicago, Ill.

- 589 HENRY, K. M., and KON, K. K.: *Vitamin B₁₂ and the biological value of proteins*, Biochem. J. 48 xi, Jan. 1951 (in Proc. Biochem. Soc.).
590. MARFATIA, U., and SREENIVASAN, A.: *Influence of vitamin B₁₂ on the biological value of low quality protein diet*, Current Sc., Bangalore 20 128-129 May 1951

591. CHOW R. F., and BARROWS L. *Role of B₁₂ on nitrogen retention of rats fed on soy bean protein diets at different caloric levels*, Federation Proc. 9 354 March 1950.

"Supplementation of vitamin B₁₂ to soy bean protein diet accelerates the rate of growth of young rats deficient of this vitamin. It is therefore of interest to ascertain whether B₁₂ enhances the efficiency of utilization of soy proteins. To this end, nitrogen balance experiments were performed to determine the amount of nitrogen retained by 2 groups of B₁₂-deficient rats, with and without supplementation of B₁₂. Their basal diet consisted of 40% sucrose and 60% Sobex, a commercial product containing 82% soy protein. Adequate vitamin supplement was also given. Urine and stool samples were collected weekly and analyzed for nitrogen, for a period of 6-8 weeks. It was found that when both groups of animals were given a restricted intake (6-8 gm. of diet/rat/day) supplementation of vitamin B₁₂ did not increase the growth rate nor the retention of nitrogen. When the dietary allowance was increased by 50% vitamin B₁₂ brought about a greater rate of growth but no better protein utilization. This is interesting since the added growth points to the effect of B₁₂ on carbohydrate or fat metabolism rather than protein metabolism. In another experiment the daily ration was further increased to ad lib feeding. The growth rate but not the efficiency of nitrogen utilization was increased by the addition of this vitamin. These results therefore indicate that B₁₂ does not enhance the biological value of soy bean proteins under our experimental conditions but may play an important role in carbohydrate or fat metabolism."

Johns Hopkins University
Baltimore, Md.

592. DACOSTA, E., and CLAYTON R.: *Serum cholinesterase of male albino rats during food restriction and subsequent protein rehabilitation*, Federation Proc. 10 879 March 1951

"Serum cholinesterase activity was measured in 182 male albino rats after 5, 10 or 20 weeks on a control, high protein (60%) low calorie or modified carrot (2% protein) diet by a decrease in pH per hour (ΔpH/hr). The average ΔpH/hr for rats on the control, high protein, low calorie or the modified carrot diet was 0.235, 0.259, 0.264 and 0.191, respectively. After 5 and 10 weeks on either the low calorie or modified carrot diet rats were rehabilitated on either a high protein diet *ad libitum* or with B₁₂ (2.5 μg/rat) or 11 gm/rat/day. Serum cholinesterase was determined after 3, 5, 7, 35 and 70 days of rehabilitation. After 18 hours of rehabilitation with the high protein diet *ad libitum* following 5 weeks on the low calorie diet a ΔpH/hr of 0.375 was found and after 10 weeks, 0.325. After 1 week of rehabilitation with a high protein diet *ad libitum* and with B₁₂ following 10 weeks on the modified carrot diet an average ΔpH/hr of 0.328 and 0.334 respectively was found, otherwise the trend was toward the control level. Since changes in serum cholinesterase activity and body weight usually parallel each other this test may serve as an estimate of the nutritive state of an animal."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

593. DACOSTA, E., and CLAYTON R.: *Serum alkaline phosphatase of male rats during food restriction and subsequent protein rehabilitation*, Federation Proc. 11 439-440, March 1952.

"Serum alkaline phosphatase activity measured in 288 male rats after 35 or 70 days on a control, high (60%) protein, low calorie, high (10%) salt, or modified carrot (2% protein) diet was 65.6, 82.9, 56.6, 114.6 and 25.3 units/100 ml. of serum, respectively. After either 35 or 70 days of restriction, the rats were rehabilitated for 3, 5, 7, 35 or 70 days on either a high protein diet *ad libitum*, or supplemented by B₁₂ (7.5 μg/rat) or 11 gm/rat/day. Serum alkaline phosphatase activity rose gradually or fell during early rehabilitation. After 7 days of rehabilitation following 35 days on the low calorie diet, serum alkaline phosphatase rose from 68 to 85% above the low calorie level and after 7 days of rehabilitation following 35 days on the carrot diet, serum alkaline phosphatase rose from 68-143%. After 7 days of rehabilitation following 35 days on the high salt diet, alkaline phosphatase was below the high salt level, but after 70 days of rehabilitation, from 30-41% above. The slowest recovery of serum alkaline phosphatase occurred in the groups given B₁₂. After 35 days of rehabilitation, it reached the control level in most rats. After 70 days, it mostly exceeded the high protein control level. A parallel rise in body weight and muscle and heart proteins suggests that serum alkaline phosphatase activity serves as a measure of nutritional status."

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Chicago, Ill.

594. CLAYTON R., and DACOSTA, E.: *Liver glycogen of the rat after food restriction and rehabilitation on a high protein diet unsupplemented or supplemented by vitamin B₁₂*, Federation Proc. 11 438, March 1952.

"The average liver glycogen of 288 male rats fed either a control high (60%) protein, low calorie high (10%) salt, or modified carrot (2% protein) diet for 35-70 days was 2.5, 2.0, 1.2, 2.0 and 4.8 gm/100 gm. of tissue, respectively. Restricted animals were rehabilitated for 3, 5, 7, 35 or 70 days. After 3, 5, 7, 35 or 70 days of rehabilitation following 85 days on the low calorie diet, the liver glycogen rose to 140 to 440% above the low calorie level. After 7 days of rehabilitation following 70 days on the low calorie diet, liver glycogen varied from 140% above to 50% below the low calorie level. After 35 days on the carrot diet, the liver glycogen with 3, 5, 7 days of rehabilitation, was reduced to 5-22% of the depletion level. After 35 or 70 days of rehabilitation following 35 or 70 days on a low calorie diet, liver glycogen was essentially at the control level. After protein depletion and subsequent rehabilitation during a similar period, liver glycogen was significantly above the control level. In early rehabilitation after caloric restriction, increased gluconeogenesis may result from increased adrenocortical activity since liver glycogen gains and increased adrenal weight paralleled each other. Increased glycogen deposition occurred later in rehabilitation following protein depletion, probably because time was needed to build up protein reserves for hormone synthesis."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

593. DIACOSTA, E., and CLAYTON, R.: *Total protein and fat of male rat carcasses after food restriction and subsequent protein rehabilitation*, *Am. J. Physiol.* 167 777 Dec. 1951 (In Soc. Proc.)

"The average total protein and fat of the carcasses of 238 rats fed either a control, high protein (60%) or 3 types of restricted diets for 5 or 10 weeks were determined. Restricted animals were rehabilitated for 14, 2, 3, 7, 35 and 70 days on either a high protein diet ad libitum, 11 gm./rat/day or plus B₁₂. After 5 weeks on 1) low calorie, 2) carrot or 3) high salt diets, total carcass protein of rats rehabilitated for 2 days on high protein ad libitum was 19.5, 23.4 and 17.1 gm. % and the fat, 9.0, 10.0, 12.6 gm. % respectively. After 2 days on high protein, 11 gm./rat/day total carcass protein was 26.7, 23.1 and 20.5 gm. % and the fat 4.3, 8.7 and 10.0 gm. %, respectively. After 2 days on high protein plus B₁₂, the total carcass protein was 24.8, 24.7 and 18.8 gm. % and the fat 4.0, 9.4 and 8.1 gm. % respectively. After 10 weeks restriction and the above pattern of rehabilitation including 10 weeks, total carcass protein and fat of rats rehabilitated on high protein plus B₁₂, or 11 gm./rat/day showed the same relatively high protein and low fat. These findings suggest that a limited protein intake or unlimited protein plus B₁₂ results in increased protein storage and decreased fat deposition."

U. S. Army Medical Research Laboratory
Chicago, Ill.

596. BOSSHARDT D. K., PAUL, W. J., and BARNES, R. H.: *The influence of diet composition on vitamin B₁₂ activity in mice*, *J. Nutrition* 40 595-604, April 1950.

A decrease in the fat level of the diet intensifies the growth retardation due to a deficiency of vitamin B₁₂. This growth retardation may be partially corrected by the feeding of fat or by the administration of vitamin B₁₂. These effects are intensified by increases in the protein level of the diet.

597. MCCOLLUM, E. B., and CHOW B. F.: *Sex differences in weight stimulating effect of B₁₂ in rats on diets of varying composition*, *Proc. Soc. Exper. Biol. & Med.* 75 20-23, Oct. 1950.

Young growing rats which were the offspring of adults on a B₁₂ deficient diet and therefore had reduced stores of this vitamin, were divided into four groups and given four diets varying in fat and carbohydrate content. Half of the animals in each group were given 0.5 mcg. of vitamin B₁₂ subcutaneously three times a week. Comparison of the average weight gains showed that on all four of the rations studied the animals that received vitamin B₁₂ gained more weight than the others. This was true of both sexes. When the calories were derived primarily from carbohydrate, vitamin B₁₂ exerted a greater effect on weight in the females than in their littermate males. The weight-stimulating effect of B₁₂ was greater in the females on high carbohydrate diets than in littermate sisters on diets of like protein content but with the calories provided by fat or a mixture of fat and carbohydrate.

Johns Hopkins University
Baltimore, Md.

598. MONSON W. J., DIETRICH, L. S., and ELVEHJEM, C. A.: *Studies on the effect of different carbohydrates on chick growth*, *Proc. Soc. Exper. Biol. & Med.* 75 256-259 Oct. 1950.

Chicks fed basal rations containing different carbohydrates grew most rapidly on dextrin, next on cereose, next on sucrose, and least on lactose. Omission of folic acid from the basal rations resulted in a decrease in growth in all cases. This decrease was least on the lactose diet, but the birds receiving dextrin still showed the best growth. This confirms the findings of Lockey et al. that the chick requires less folic acid on rations containing dextrin than on rations containing sucrose.

Daily injections with a low level of vitamin B₁₂ (0.1 mcg.) gave slight responses with all carbohydrates tested; higher levels (0.6 and 1.0 mcg.) gave greater responses with lactose than with the other carbohydrates. The lactose-fed birds also gave the greatest response to reticulin and fish solubles. Neither these substances nor cellulose and sulfazidine changed the significant differences observed when the different carbohydrates are fed. Dextrin was retained longest in the body than sucrose, and lactose. The possibility of these nutritional differences being explained by the synthesis of known and/or unknown factors is discussed.

University of Minnesota
Madison, Wis.

599. LING, C. T., and CHOW B. F.: *Observations on some biochemical changes induced by administration of vitamin B₁₂ to deficient animals*, *Federation Proc.* 10 216, March 1951.

"Although our knowledge of the metabolic role of vitamin B₁₂ is meager experimental results point to its possible relationship to carbohydrate or fat metabolism. In order to throw some light on this subject, experiments were performed so as to follow some biochemical changes induced as a result of administering vitamin B₁₂ to deficient animals. Rats from parents raised on soybean diet were used in all experiments. Each series of experiments consisted of a deficient and a B₁₂-injected group. Vitamin B₁₂ was given subcutaneously to the latter in dosages ranging from 25 milligrams/day to 0.5 gamma 3 x a week for as long as 12 weeks. During the period of injection, blood sugar levels after varying period of fasting, were determined on both groups of animals. Blood sulfhydryl content was measured by the amperometric titration method. At the end of the experimental period, the animals were bled by cardiac puncture and sacrificed. Glycogen, fat, and nitrogen content of the liver and total nitrogen, fat, and muscle glycogen content of the carcasses were analyzed. The results show that the deficient animals had much lower body fat, and higher water content, and lower blood sulfhydryl content, as compared to their litter mates on the same diet but receiving B₁₂ injection. The significance of these findings is discussed in the light of the role of vitamin B₁₂ in carbohydrate, fat, and protein metabolism."

Johns Hopkins University
Baltimore, Md.

600. LING, C. T., and CHOW B. F.: *Effect of vitamin B₁₂ on the body composition of rats*, *J. Biol. Chem.* 198 439. Sept. 1952 (abstr. *J. Am. Dietet. A.* 29 58, Jan. 1953.)

"It has been suggested that vitamin B₁₂ may be involved in the utilization of carbohydrates and their transformation to fat rather than in protein metabolism. To study the theory further weanling young of vitamin B₁₂-deficient female rats were maintained on diets deficient in the vitamin, some being given crystalline vitamin B₁₂ subcutaneously. Administration of the vitamin enhanced the growth rate and increased the food intake. The bodies of animals with vitamin B₁₂ deficiency were shown to have low fat, high water and normal protein contents. Upon chemical analyses, livers of the control and injected groups showed no significant differences in percentage composition of glycogen, nitrogen, or fat. However, in the deficient animals the body weight was smaller relative to the liver weight in comparison with the injected controls. These results appear to substantiate the original hypothesis that there is a relationship between B₁₂ and carbohydrate metabolism."

MILK STUDIES

601. ANNOTATION *X* nutrient identified as Vitamin B₁₂, News Edition (Chem. & Engin.) 27 1818, May 2, 1949

The hitherto unidentified food material in milk that was first reported in 1932 by the Bureau of Dairy Industry and called nutrient X because of its unknown chemical nature, is undoubtedly vitamin B₁₂, the U. S. Department of Agriculture has announced.

602. CUTHBERTSON W. F. J., and THORNTON D. M. The effect of dietary lactose on the response of the rat to vitamin B₁₂, Brit. Med. J. 5 xii, 1951.

Marked depression of growth was observed in weanling rats fed a soya diet containing 20% lactose. This effect was completely overcome by 80 to 40 mcg. of vitamin B₁₂ orally per week.

The amounts of vitamin B₁₂ required to produce significant growth increments were such as to make impracticable vitamin B₁₂ assay based on its growth-promoting effects for these rats.

603. COLLINS, R. A., DIETRICH, L. S., and ELVEHJEM, C. A.: Significance of vitamin B₁₂ in milk diets, Federation Proc. 9 355, March 1950.

"The inferior growth observed in rats fed goat's milk mineralized with iron, copper and manganese as compared to the good growth of rats fed mineralized cow's milk was studied. This inferior growth was counteracted by a daily addition to the mineralized goat's milk diet of 50 µg. of folic acid plus 0.1 µg. of vitamin B₁₂, 0.5 µg. vitamin B₁₂ alone, or 1.0 Gm. of fresh beef liver. An inconsistent half maximal growth stimulation resulted when 50 µg. of folic acid or 0.1 µg. of vitamin B₁₂ was added separately to this diet. Goat's milk was found to contain only traces of vitamin B₁₂ in contrast to the occurrence of 2-3 µg. of vitamin B₁₂/L in cow's milk. The folic acid content of goat's milk was found to be very low and comparable to cow's milk. Folic acid or vitamin B₁₂ additions to mineralized cow's milk diets did not affect growth in the young rat. The addition of vitamin C to the cow's milk diets produced a large increase in the vitamin B₁₂

content of the livers, while on the goat's milk diets added vitamin C displayed no activity. Folic acid and vitamin B₁₂ additions to mineralized cow's milk or goat's milk did not affect the rate of hemoglobin formation in the weanling rat or in rats made anemic on milk diets."

604. BUREAU OF DAIRY INDUSTRY. Vitamin B₁₂-active materials in milk aid in utilization of protein, Report of the Chief of the Bureau of Dairy Industry U. S. Department of Agriculture, 1950 (cited in J. Am. Dietet. A. 27 120, Feb. 1951)

"In investigations of the sources of vitamin B₁₂ in milk, the Bureau of Dairy Industry U. S. Department of Agriculture, found that some rats can synthesize vitamin B₁₂-active materials microbiologically when they are on rations consisting almost entirely of plant products. These results tend to support the view expressed previously which was based on strong circumstantial evidence, that the cow also is able to synthesize vitamin B₁₂-active materials which she secretes in her milk.

"Other phases of the study showed that vitamin B₁₂ is effective in improving diets that are low in protein, as well as being effective in enabling rats to use rations that contain high levels of protein. Without a source of vitamin B₁₂, high levels of protein were found to be very harmful, and even fatal.

"Vitamin B₁₂ was also shown to be essential for normal growth of rats when the ration was high in lactose (milk sugar). The depressing effect on growth, when the diet contains lactose but lacks vitamin B₁₂, appears to be due, in part at least, to suppression of microbiologic synthesis of this vitamin in the rat."

605. COLLINS, R. A., HARPER, A. E., SCHREIBER, M., and ELVEHJEM, C. A. Folic acid and vitamin B₁₂ content of milk of various species, J. Nutrition 43 313-321, Feb. 1951.

HYPERHYPOTHYROIDISM: VITAMIN B₁₂ IN

606. NICHOL, C. A., DIETRICH, L. S., GRAYENS, W. W., and ELVEHJEM, C. A.: Activity of vitamin B₁₂ in the growth of chicks, Proc. Soc. Exper. Biol. & Med. 70 40-42, Jan. 1949

Experiments are reported which indicate that vitamin B₁₂ is highly active in stimulating the growth of the hyperthyroid chick. Hyperthyroidism was induced by feeding a basal ration containing 0.05% iodinated casein. Supplementation of this diet with 3% fish solubles produced in a two week period an average gain over the unsupplemented group of 86 Gm. per chick. Vitamin B₁₂ at the level of 0.75 mcg./100 Gm. of ration resulted in a gain of 47 Gm., and 1.5 mcg./100 Gm. produced a gain of 88 Gm. The gain in weight over the unsupplemented group was 86 Gm. when reticulogon was given intravenously in the dosage of 0.5 U.S.P. unit per bird per day. Daily intravenous injection of 0.01 mcg. vitamin B₁₂ gave a growth response of 21 Gm., 0.1 mcg. a response of 72 Gm., and 0.5 mcg. a response of 79 Gm. It is concluded that pure vitamin B₁₂ can replace the animal protein factor activity of condensed fish solubles and injectable liver preparations.

- 607 BOSSHARDT D. K., PAUL, W. J., O'DOHERTY K., HUFF, J. W., and BARNES R. H.: *Mouse growth assay procedures for the "animal protein factor"* J Nutrition 37 21-35, Jan. 1949

The "animal factor" has been assayed by two methods, one of which involves the use of growing mice born of mothers that were maintained on a diet free of this factor while the other is based on the ability of the "animal protein factor" to counteract the growth retardation of mice that are fed materials having thyroid activity. Studies showed that liver contains a factor or factors essential for growth not present in yeast or wheat germ, and not identical with any known vitamin factor. This factor counteracts the growth retardation of mice that are fed thyroid-active materials. It may be transmitted from the mother to the young during gestation or lactation or both and may be stored by the animal for a considerable length of time. A severe lack of the "animal protein factor" in the maternal diet and tissue stores results in a pronounced mortality of young mice one to three days post partum. The possibility is suggested that the "animal protein factor" may be identical with vitamin B₁₂.

608. EMERSON G.: *Growth-promoting activity of vitamin B₁₂ in rats receiving thyroid substance* Proc. Soc. Exper. Biol. & Med. 70 392-394, March 1949

The growth of rats on a soybean meal diet was greatly retarded by the addition of 0.25% thyroid powder. The daily addition of 0.125 mcg vitamin B₁₂ resulted in a weight gain exceeding that of rats receiving the soybean meal without thyroid, and approximating that of rats on a casein diet. A lower level of vitamin B₁₂ reduced the gain, but higher levels did not greatly increase it. Cobalt did not increase the weight gain over that of the controls, which indicates that cobalt could not be employed under these conditions in the biosynthesis of vitamin B₁₂.

March Institute for Therapeutic Research
Baltimore, Md.

- 609 ERSHOFF R. H.: *Beneficial effects of liver on growth and survival in the immature hyperthyroid mouse*, Proc. Soc. Exper. Biol. & Med. 70 898-901 March 1949

Whole liver powder prolonged survival and counteracted the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxine, or iodinated casein. The protective factor(s) was apparently distinct from any of the known nutrients and was retained in the water-insoluble extracted liver residue.

Contrary to the findings of Boshardt et al. (Abstr 607) Wilson's liver concentrate 120 did not increase the growth or survival of mice fed iodinated casein or desiccated thyroid, but did promote the growth of mice fed thyroxine although it was less effective in this respect than extracted liver residue. Since liver concentrate 120 contains vitamin B₁₂, factors other than this vitamin apparently are required by the hyperthyroid mouse. A multiple dietary deficiency may be precipitated in animals on purified rations when they are made hyperthyroid. Whether the first limiting factor is a deficiency of vitamin B₁₂, or the antithyrototoxic factor(s) of extracted liver residue or of still other nutrients appears to depend

on such factors as the pre-test dietary regimen, the composition of the diet employed, bacterial synthesis, or strain and species differences in nutritional requirements.

610. BETHEL, J. J., and LARDY H. A.: *Comparative effectiveness of vitamin B₁₂, whole liver substance and extracts high in APA activity as growth promoting materials for hyperthyroid animals*, J Nutrition 37 495-509 April 1949

In thyrotoxicosis, rats require one or more growth factors which are not needed by normal rats fed highly purified diets. Purified liver extracts rich in the anti pernicious anemia (APA) factor are a good source of this growth factor(s). The growth response may be due to some constituent other than the APA factor since the thyrotoxicosis produced by the thyroid-containing ration is not accompanied by an anemia and the hemoglobin levels of animals showing the typical growth depression are normal. However on a dry weight basis liver fractions containing less APA activity are also less active in supporting the growth of hyperthyroid rats. If this growth stimulating factor is the same as that required for blood cell maturation in human beings, these observations may serve as the basis for an animal assay for the APA factor. The graded growth response obtained with increasing doses of purified liver extracts indicates the possibility of such an assay.

The effect of APA preparations was also tried in rabbits. During a six week period of thyroxine administration, 6 of 7 control animals died, while 6 of 7 that were given purified liver extract for the last four weeks of the period survived although they did not grow.

The intraperitoneal administration to hyperthyroid rats of 0.2 mcg. crystalline vitamin B₁₂ daily or the addition of 30 mcg. B₁₂ per Kg. of ration, promoted growth as well as did the liver extract. Thus it seems that all or most of the growth-promoting activity of APA liver extracts resides in their vitamin B₁₂ content. It appears that the vitamin B₁₂ requirement of the hyperthyroid rat lies between approximately 0.1 and 0.2 mcg. per day when the vitamin is administered parenterally.

Exhaustive extraction of whole liver powder with ethanol, ethanol-ether or water brought only part of the active material into solution, which is another indication of the possible dual nature of the material which, in addition to the known vitamins, supports the growth of animals fed thyroxine-containing rations.

Treatment of folic acid with good sources of the "intrinsic factor" did not result in the formation of a growth-promoting substance, and "formylfolic acid" was likewise ineffective.

- 611 ERSHOFF R. H.: *An antithyrototoxic factor for the rat not identified with vitamin B₁₂*, Proc. Soc. Exper. Biol. & Med. 71 209-211, June 1949

On purified rations containing casein as the dietary protein and sucrose as the dietary carbohydrate, the growth of hyperthyroid rats was reduced. The retardation of growth was completely counteracted by the administration of a water insoluble fraction of liver, but crystalline vitamin B₁₂ was ineffective. The protective factor in liver is distinct from any of the known nutrients, including vitamin B₁₂.

612. ERSHOFF B. H. *Protective effects of soybean meal for the immature hyperthyroid rat*, J. Nutrition 39 259-281, Oct. 1949

Full-fat soybean meal completely counteracted the growth retardation of immature rats fed massive doses of desiccated thyroid or iodinated casein. The retardation in growth of immature hyperthyroid rats on a diet containing low-fat soybean flour was completely counteracted by the administration of either soybean oil, extracted liver residue or crystalline vitamin B₁₂. When immature rats were fed a similar diet but containing casein as the dietary protein, the growth retardation following thyroid feeding was completely counteracted only by extracted liver residue, vitamin B₁₂ being inactive.

University of Southern California
Los Angeles, Calif.

613. ERSHOFF B. H. *Effects of vitamin B₁₂ and liver residue on growth of hyperthyroid male rats*, Proc. Soc. Exper. Biol. & Med. 73 459-461, March 1950.

Massive doses of thyroid in conjunction with a purified diet containing casein as the dietary protein resulted in retardation in both body and gonadal weight in immature male rats. These effects were completely counteracted by the administration of liver residue. Crystalline vitamin B₁₂, however, at a level of 30 mcg. per Kg. of diet, was without significant effect. The growth retardation and inhibition of testicular development is apparently due to a deficiency of some factor other than vitamin B₁₂.

University of Southern California
Los Angeles, Calif.

614. LEWIS U. J., TAPPAN D. V., REGISTER U. D., and ELVEHJEM, C. A. *Effect of carbohydrate on growth response to vitamin B₁₂ in the hyperthyroid rat*, Proc. Soc. Exper. Biol. & Med. 74 566-571 July 1950.

Studies were undertaken to elucidate the nature of antithyroidic effects of various carbohydrates in relation to the specificity of the rat assay for vitamin B₁₂. Rats on a sucrose-casein diet containing a thyroid-active material do not respond completely to vitamin B₁₂, but this can be overcome by substituting defatted corn meal, corn starch or dextrin for the sucrose. This may possibly be attributed to the intestinal synthesis of other required factors.

Growth depression in the hyperthyroid rat was counteracted to a slight extent by the addition of corn oil to the corn-soybean ration containing iodinated casein, but this may have been due to the fact that these animals consumed less food and, therefore, less iodinated casein.

University of Tennessee
Knox, Tenn.

615. PENTZ, E. L., GRAHAM, C. E., RYAN, D. E., and KLEIN, D. *The ability of liver preparations and vitamin B₁₂ to maintain thymus weight in thyroid fed rats having greatly hypertrophied adrenal glands*, Endocrinology 47 30-35, July 1950.

A study is described in which it was found that vitamin B₁₂ has a protective effect on the thymus in rats fed thyroid. Rats bred on a ration containing soybean meal, dextrose, and vegetable fat, supplemented with ex-

cess amounts of all the crystalline vitamins, were fed this diet plus 0.25 per cent desiccated thyroid with and without supplements of injectable liver preparations, a vitamin B₁₂ concentrate and crystalline vitamin B₁₂.

Autopsy studies revealed that the rats receiving desiccated thyroid alone had greatly enlarged adrenal glands and atrophy of the thymus. Rats which received liver preparations, the vitamin B₁₂ concentrate, and crystalline vitamin B₁₂ in addition to the desiccated thyroid, had equally large adrenals but did not show atrophy of the thymus. One of the functions of vitamin B₁₂ in bacterial metabolism is in the synthesis of the thymine deoxy riboside. It has been shown previously that the two areas containing the highest concentration of newly formed deoxyribonucleic acids are the thymus and the crypts of Lieberkuhn. This may provide the basis for an explanation of the protective effect of vitamin B₁₂ on thymus weight in the present experiments.

The Wistar Laboratories
Chicago, Ill.

616. MEITES, J. *Effects of vitamin B₁₂ on thymic action in rats*, Proc. Soc. Exper. Biol. & Med. 75 193-195, Oct. 1950.

Author's summary: "The effects of crystalline vit. B₁₂ on thymic action was determined in immature female rats for a 30-day period. The vitamin completely counteracted the growth-inhibiting action of thiouracil, and this was accompanied by a considerable increase in food consumption. Although vit. B₁₂ decreased the thyroid hypertrophy induced by thiouracil, the uptake of radioactive iodine (I¹³¹) by the thyroids was even less than in the rats which received thiouracil only. It is suggested that vitamin B₁₂ may be able to induce normal growth in hypothyroid rats."

Washington State College
East Lansing, Mich.

617. MEITES, J. *Effects of vitamin B₁₂ on normal thyroid function in rats*, Proc. Soc. Exper. Biol. & Med. 75 195-197 Oct. 1950.

Author's summary: "Crystalline vit. B₁₂ was fed to immature rats of both sexes in order to determine whether the vitamin could alter thyroid function in normal or thyroprotein-treated rats. The growth rate of the rats supplemented with the vitamin was increased above that of the normal or thyroprotein-treated controls, but there was no significant effect on thyroid weight or uptake of I¹³¹. It is concluded that vit. B₁₂ does not alter normal thyroid activity in rats."

Washington State College
East Lansing, Mich.

618. BOLENE, C., ROSS, O. B., and MACVICAR, R.: *The growth promoting action of various supplements in the hyperthyroid rat*, Proc. Soc. Exper. Biol. & Med. 75 610-613, Nov. 1950.

The reversal of growth inhibition induced by feeding thyroid-active substances to the immature rat has been used by other workers as an assay procedure. In the experiments reported here, rats receiving toxic levels of iodinated casein were given various supplements to counteract the effect of the iodinated casein on growth.

Fish solubles, 1.5 per cent in the diet, two commercial APP supplements and 1:20 liver powder all of which have appreciable animal protein factor activity for the chick, proved effective in counteracting the inhibition of growth by iodinated casein. Crystalline vitamin B₁₂ gave a highly significant growth response in males, but was less effective in females. Even in males it did not produce maximum response. This suggests that the "antithyroid factor" is multiple in nature and that vitamin B₁₂ is one of the components.

Oklahoma Agricultural and Mechanical College
Stillwater, Okla.

- 619 RUPP J., PASCHKIS, K. E., and CANTAROW A.: Influence of vitamin B₁₂ and liver extract on nitrogen balance of normal and hyperthyroid rats. *Proc. Soc. Exper. Biol. & Med.* 76 432-435, March 1951

Vitamin B₁₂ (3 mg twice daily) or liver extract (0.2 cc.) did not induce gain in weight nor nitrogen retention in force-fed rats on constant food intake. Injection of thyroxine produced identical weight losses in the rats given vitamin B₁₂ and in the controls, although the nitrogen loss resulting from the catabolic action of thyroxine was significantly less in the treated animals. Liver extract had a similar action, but the values obtained were not statistically significant. These results suggest that in hyperthyroid rats, vitamin B₁₂ spares protein at the expense of other body constituents.

Rosen-Emery Institute for Cancer Research
New York, N. Y.

620. RUPP J.: Action of vitamin B₁₂ and of liver extract on nitrogen balance of hyperthyroid rats. *Federation Proc.* 10 115, March 1951.

"Considerable experimental evidence has accumulated during the past several years showing that the weight loss of hyperthyroid animals can be prevented, and even normal growth of such animals maintained, if vitamin B₁₂ or liver preparations are administered. This effect is largely due to stimulation of appetite induced by the supplements, but it has been suggested that in addition food utilization is improved. We have performed experiments on force-fed rats fed a constant amount of a diet containing about 16% protein derived from yeast and milk powder. Neither vitamin B₁₂ (6 γ /day) nor liver extract (0.4 U.S.P. U — 0.2 ml./day) caused any change of weight or of N excretion. Rats fed in this way and made hyperthyroid by daily injection of 150 γ of *d,l* thyroxine, lost weight and exhibited increased N excretion. When such thyroxine-treated rats were given vitamin B₁₂, the thyroxine induced weight loss was not changed, but the N loss was significantly decreased. Since it is known that the calorific effect of thyroxine is not counteracted by the vitamin and since the weight loss of the hyperthyroid rat was not influenced by B₁₂ or liver extract, it would appear that the latter are N sparing at the expense of material other than protein."

Jefferson Medical College
Philadelphia, Pa.

- 621 MEITES J.: Counteraction of anabolic effects of mild hyperthyroidism by vitamin B₁₂. *Federation Proc.* 10 91 March 1951.

"Vitamin B₁₂ has been shown to counteract the growth-retarding effects induced in rats by administering thyroid-active materials. Young mice, unlike rats, respond to the administration of low doses of thyroid substances by true increases in growth rate, and it was of interest to determine whether vitamin B₁₂ could modify this effect. Forty immature albino mice (Rockland) weighing approximately 13.0 gm. each, were divided into 4 uniform groups and fed a basal ration low in vitamin B₁₂ for 30 days. The following substances were added to the ration of each group: 1) controls, nothing; 2) 0.025% thyroprotein (Cerophyl); 3) 100 μ g. of vitamin B₁₂ (Merck) per kg. of ration; 4) 0.025% thyroprotein and 100 μ g. of vitamin B₁₂. The total average gain in body weight and daily average food consumption in grams per mouse were as follows for each group: 1) 9.6 and 3.2; 2) 18.5 and 5.4; 3) 15.9 and 4.0; 4) 10.6 and 4.0. It can be seen that either thyroprotein or vitamin B₁₂ alone considerably increased body weight and food intake above the controls, while the combination of the two substances eliminated the favorable effects of either on body growth. The efficiency in converting food into body gains was greatest for the groups receiving thyroprotein or vitamin B₁₂ alone, and least for the group receiving both substances."

Michigan State College
East Lansing, Mich.

622. GREER, M. A.: Failure of vitamin B₁₂ to modify goitrogenic action of thiouracil. *Proc. Soc. Exper. Biol. & Med.* 77 146-147 May 1951

The development of goiters in rats fed thiouracil was not affected by the addition to the diet of an aqueous solution of vitamin B₁₂ or of crystalline B₁₂ as a tritrate of sodium chloride. This is contrary to the findings reported by Meltes (*Proc. Soc. Exper. Biol. & Med.* 75 193, 1950 [Abstr. 616])

Free England Cancer Hospital, and
Trinity College Medical School
Durham, Mass.

- 623 WATTS, A. B., ROSS, O. B., WHITEHAIN, C. K., and MACVICAR, R.: Response of castrated male and female hyperthyroid rats to vitamin B₁₂. *Proc. Soc. Exper. Biol. & Med.* 77 624-626, Aug. 1951.

Because of conflicting reports regarding the influence of sex on response to vitamin B₁₂, the growth promoting effect of this vitamin in the hyperthyroid rat was studied in normal and castrated rats of both sexes. Growth depression was produced by feeding toxic levels of iodinated casein. Vitamin B₁₂, 25 mg. per Kg., was less effective than liver concentrate powder 1:20, in counteracting growth depression in normal males and in oophorectomized females. There was little difference in response to these supplements in normal females and castrate males. It is suggested that the male sex hormone may act synergistically in the hyperthyroid rat with some factor present in liver and not identical with vitamin B₁₂, or that the female sex hormone is antagonistic to such a factor.

Oklahoma Agricultural Experiment Station
Stillwater, Okla.

- 624 DRYSDALE, G. R., BETHEIL, J. J., LARDY, H. A., and BAUMANN, C. A.: The excretion of the citrovorum factor by hyperthyroid rats. *Arch. Biochem. & Biophysics* 33 1-8, Aug. 1951.

In immature rats fed diets containing folic acid and 0.25% of desiccated thyroid, the administration of vitamin B₁₂ or of whole liver powder caused an increased urinary excretion of the citrovorum factor

625. BARKER, S. B., DIRKS, H. B., JR., KLITGAARD H. M., and GARLICK, W. R. *Inhibition of metabolic action of thyroxine*, Am. J. Physiol. 167: 765, Dec. 1951 (In Soc. Proc.)

"Using the thyroidectomized rat maintained on 12 µg. m-thyroxine as a test animal, we have continued the investigation of the capacity of substances to interfere with the metabolic action of the hormone. Molecular ratios of compound to thyroxine have been computed on the basis of the quantities injected, assuming no endogenous source of thyroxine. Synthetic vitamin A, both as the solution in oil and saline suspension of the palmitate, exhibited inhibiting effects of 20-40% at molar ratios of 300-2000. Folic acid was inactive at 500x, but B₁₂ showed a 21% effect at 10x. The 5-iodothioracil and 3-fluoro-4-hydroxyphenylacetic acid both depressed thyroxine action about 40% at doses 500x isomolar to that of the hormone. Several members of a series of organic acids containing a 3,5-diiodo-4-hydroxyphenyl substitution displayed thyroxine-inhibiting qualities. Various derivatives of 3,5-diiodo-4-hydroxybenzoic acid also were active antagonists, although 3,5-diiodotyrosine exhibited some thyroxine-like action."

From University of Iowa
Iowa City, Iowa

626. ARCHER, J. D., NASH, J. B., BROWN R. G., and EMBERTSON G. A. *Failure of excess vitamin B₁₂ in influencing metabolic and toxic effects of thyroid U.S.P. XIV* Federation Proc. 11: 318, March 1952.

"Effects of vitamin B₁₂ on viability and growth of thyroid- or thyroxine-treated rats deficient in this vitamin have been noted by several authors none describes actions of vitamin B₁₂ in modifying metabolic effects of large doses of thyroid substance in rats on a relatively adequate diet. Forty five young female rats were divided into 3 groups of ca. the same mean body weights, within 5% and maintained on Purina dog chow. Thyroid was given intragastrically for 16 days in 2 groups, as a suspension of the finely powdered drug, in a dose of 450 mg/kg. one of these groups also received 16 daily doses of 75 µg/kg. of vitamin B₁₂, intraperitoneally. O₂ consumption, estimated manometrically and body weights were noted during the last 8 days of treatment. No significant differences in either metabolic rate or weight were noted between the 2 groups receiving thyroid, but there was a substantially increased metabolic rate and a failure of growth in both treated groups as compared with the untreated controls. Similar results were found with thyroxine."

University of Texas Medical Branch
Galveston, Texas

627. GRAHAM, C. E., REICHSTEIN I. P., WATSON W. J., and HIER, E. W. *Effects of liver fractions and vitamin B₁₂ on body organ weights of thyroid fed rats*, Proc. Soc. Exper. Biol. & Med. 80: 657 659 Aug.-Sept. 1952.

In experiments designed to show the effect of thyroid, defatted liver residue, liver concentrate N F., and vitamin B₁₂ on organ weights of young rats, vitamin B₁₂ was without effect except in preventing hypertrophy of the spleen due to thyroid feeding. Lycopodium was found to have no antithyroidic effect in the diet used.

University of Rochester School of Medicine and Dentistry
Rochester, N. Y.

628. PALMER, J. G., CARTWRIGHT G. E., and WIN TROBE, M. M.: *Experimental production of leukopenia in rats by thyroid feeding*, Am. J. Physiol. 171: 585-590, Nov. 1952.

Authors summary: "Rats fed desiccated thyroid and a diet limited in caloric value developed severe leukopenia and lost weight. The leukopenia involved both granulocytes and mononuclear cells and was not accompanied by anemia. It was not corrected by increasing any one of the constituents of the diet nor by giving vitamin B₁₂, folic acid, citrovorum factor, ascorbic acid, lycopodium, whole liver powder, vegetable fat or yeast. Evidence is presented which suggests that the leukopenia is related to a limited caloric intake."

University of Utah College of Medicine
Salt Lake City, Utah

629. OVERBY L. R., FREDERICKSON R. L., and FROST D. V. *The antithyroidic factor* Federation Proc. 12: 425 March 1953

"A water-insoluble liver fraction has been found by Ershoff (Proc. Soc. Exp. Biol. and Med. 64: 500, 1947) to counteract the growth retardation of immature rats fed massive doses of thyroxine. The present study concerns assay for this factor using procedures based on the work of Ershoff and the assay in rats for vitamin B₁₂ in this laboratory. Responses to vitamin B₁₂ or to liver residue differ depending on the level of thyroxine fed. A synthetic type sucrose, casein diet containing vitamin B₁₂ was developed. The most critical assay for the antithyroidic factor was obtained with 0.3% iodinated casein as the thyrotoxic agent. Weanling male rats were depleted for 10 to 12 days on the basal ration without thyroxine. Maintenance of a near normal growth rate and decrease in mortality were taken as criteria of the antithyroidic activity of supplements to the basal diet. Liver residue (5-10%) partially counteracts the thyrotoxic effects. Other substances studied were synthetic and natural fats, fibrin, hydrolyzed fibrin, egg albumin, defatted whole egg lecithin, lycopodium, lipolic acid, ACTH, orotic acid, adenine and guanine. Of these, only fats and defatted whole egg showed protective action against thyrotoxicosis. Isolation of the active principle in liver residue is under study."

Abraham Laboratories
North Chicago, Ill.

OTHER HORMONES

630. METTES, J., and NEWLAND H. W.: *Effects of vitamin B and thyroprotein on growth-inhibiting action of diethylstilbestrol in rats* Federation Proc. 9: 87-88, March 1950.

"Growth depression induced by diethylstilbestrol in rats has been accounted for on the basis of reduced appo-

titis (Meites, *Am. J. Physiol.* 159 281 1919) It was hypothesized that these inhibitory effects were induced by increasing certain vitamin needs and/or decreasing thyroid secretion. This hypothesis was tested by treating 5 evenly divided groups of 50 male rats for 20 days. Diethylstilbestrol and thyroprotein were fed in a commercial ration, while B_{12} was injected subcutaneously at a level of 0.2 mg. daily. During the first 10 days, B_{12} failed to counteract the growth depression induced by diethylstilbestrol, but partially overcame the effects of the latter during the last 10 days. Thyroprotein plus diethylstilbestrol-thyroprotein combination completely counteracted the growth-inhibiting action of the latter and only slightly that of the former. Food intake was increased both by B_{12} and thyroprotein."

Washington State College
East Linn, Wash.

631. MEITES, J., and SHAY J. C. Effects of vitamin B_{12} on diethylstilbestrol and thyroprotein action in male rats, *Proc. Soc. Exper. Biol. & Med.* 76 196-198, Jan. 1951

The effects of crystalline vitamin B_{12} on several actions of diethylstilbestrol and thyroprotein were determined in 100 young male albino rats. Diethylstilbestrol and thyroprotein were incorporated into the ration in amounts of 0.10 and 0.16 per cent, respectively and vitamin B_{12} was injected subcutaneously in doses of 0.2 or 0.4 mg. daily. Vitamin B_{12} partially counteracted the growth-retarding action of these two hormones when given separately or together and also increased food consumption. The vitamin did not alter the ability of the hormones to depress testes weight or the ability of diethylstilbestrol to increase the weight of the seminal vesicles.

An addendum states that data obtained since the submission of this manuscript indicate that vitamin B_{12} probably does not alter the turnover of thyroprotein in the rat. The increase in the basal metabolism of rats given thyroprotein was not affected by the administration of vitamin B_{12} .

Washington State College
East Linn, Wash.

632. MEITES, J. Changes in nutritional requirements accompanying marked changes in hormone levels, *Metabolism* 1 58-67 Jan. 1952.

Author's summary "Large doses of diethylstilbestrol or thyroprotein, fed to young male rats on an adequate ration, severely inhibited body growth. Supplementation of the ration with vitamin B_{12} partially counteracted growth inhibition and increased appetite. The decrease in testes weight which resulted from the hyperestrinism or hyperthyroidism, and the increase in weight of the seminal vesicles by the oestrogen, were not influenced by vitamin B_{12} .

"The inhibition of body growth in young female rats by thyroprotein was completely counteracted by incorporation of small amounts of procaine penicillin G or neomycin sulfate in an otherwise adequate diet. Streptomycin sulfate was ineffective in this respect. The large increase in basal oxygen consumption (74%) induced by thyroprotein was not affected by the antibiotics.

"Of 18 young male rats on a vitamin B_{12} -deficient ration subjected to thyroproteinectomy 15 survived for 30 days, 5 for 60 days, and none for 90 days. When the diet was supplemented with vitamin B_{12} , 21 out of 26 survived for 30 days, 19 survived for 60 days, 17 for 90 days, and 16 for 120 days. The vitamin enabled growth to proceed in the surviving rats, although at a considerably slower rate than in intact rats given vitamin B_{12} .

"Large daily injections of cortisone inhibited body and hair growth in young male rats fed a ration deficient in vitamin B_{12} . The addition of vitamin B_{12} , either alone or together with streptomycin hydrochloride, to the diet completely counteracted inhibition of body growth and partially of hair growth. The vitamin and antibiotic completely protected the thymus against atrophy by cortisone but did not prevent the decrease in adrenal weight.

"It is concluded that (a) marked changes in thyroid, estrogen, or cortisone levels in the body can increase requirements for vitamin B_{12} ; and possibly other dietary factors in rats (b) supplementation of diets with vitamin B_{12} and antibiotics can partially or completely prevent manifestations of dietary deficiencies induced by these hormones and (c) vitamin B_{12} and the antibiotics do not alter certain characteristic actions of these hormones in the body with the exception that cortisone-induced atrophy of the thymus can be prevented by vitamin B_{12} and streptomycin."

Washington State College
East Linn, Wash.

633. DACOSTA, E., and CLAYTON R. Histological and biochemical changes in the rat adrenal after dietary restriction and rehabilitation, *Am. J. Physiol.* 171 717 Dec. 1952 (in Soc. Proc.)

"The weight, total cholesterol, vitamin C, sudanophilic and birefringent material of the adrenal and eosinophil count were determined on 288 rats after 85 days of either 1) caloric restriction, 2) a 2% protein diet, 3) a 4.5 protein and 10% salt diet and after 85 days of rehabilitation on either 4) a 60% protein ad libitum, 5) the same, 11 gms./day or 6) the same plus B_{12} . In the calorically restricted or high protein controls less birefringence occurred in all zones. Increased sudanophilic or birefringent material was found in the fasciculi after diets 2 and 3. During rehabilitation after caloric restriction sudanophilic and birefringent materials decreased in the reticula and increased in the glomerulosa. During rehabilitation after diet 2, the sudanophilic distribution resembled the control while birefringence increased in the fasciculi. Following rehabilitation after diet 3, sudanophilic and birefringent material was decreased in the glomerulosa but increased in the fasciculi and reticula. Usually a reciprocal relationship existed between adrenal weight and number of eosinophils and between vitamin C and cholesterol content. The adrenals of the high protein controls weighed 50.2 mg., eosinophils 0.55% of total white cells, cholesterol 0.65 mg. % vitamin C 0.24 mg. % compared to 45.2 mg., 1.09% 2.4 mg. % and 0.18 mg. % for the normal controls and 24.0 mg., 1.51% 1.0 and 0.07 mg. % after diet 5 respectively. Thus restriction and rehabilitation evidently involve adaptation partly controlled by the adrenals."

U. S. Army Medical Nutrition Laboratory
Chicago, Ill.

- 634 GERSHBERG H., and RALLI, E. P. *Adrenal changes associated with a low protein diet*, Federation Proc. 11: 54, March 1952.

"It is established that a low protein-high fat diet will produce fatty infiltration of the liver. Other tissues probably also suffer from this protein deficiency. In particular the pituitary which secretes protein hormones. In rats fed such a diet (9% protein and 38% fat) the adrenals and the thymus were weighed and determinations were made of the adrenal cholesterol, liver fat and liver nitrogen after periods of 8 to 60 days on the diet. The adrenal cholesterol was increased about 2 g.% after only 8 to 12 days on the diet. After 54 days, the adrenal cholesterol was further elevated and the adrenal weight had diminished. These changes are in the same direction as those observed in hypophysectomized rats, though not as pronounced. The increase in adrenal cholesterol was not associated with an increase in liver brain or serum cholesterol which in most cases was within normal limits. Administration of ACTH (2 mg. daily) caused an increase in adrenal weight and a decrease in thymus weight, indicating that the adrenals were not refractory. Vitamin B₁₂ (5 mcg. daily) had a distinct lipotropic effect on the liver within a period of 7 to 12 days, but the adrenal cholesterol was not reduced."

University of New York College of Medicine, and
Bellevue Hospital Center
New York, N. Y.

635. RUPP J., and PASCHKIS, K. E. *Lack of influence of vitamin B₁₂ on protein catabolic action of cortisone*, Proc. Soc. Exper. Biol. & Med. 73: 65-66, Jan. 1953.

Authors summary "In rats receiving a constant food intake by tube feeding, the weight loss and N loss induced by cortisone is not improved by treatment with vitamin B₁₂. This failure of vitamin B₁₂ to influence cortisone induced protein catabolism is in contradistinction to the N sparing effect of vitamin B₁₂ in thyroxine induced protein catabolism."

Jefferson Medical College
Philadelphia, Pa.

ANTIBIOTIC SUPPLEMENTS

Chicks

636. STOKSTAD E. L. R., and JUKES, T. H. *Further observations on the "animal protein factor"* Proc. Soc. Exper. Biol. & Med. 73: 523-528, March 1950.

Chicks with a low reserve of the animal protein factor were kept on experimental diets adequately supplied with vitamin B₁₂, either in the crystalline form or as a mixture of vitamin B₁₂ and vitamin B_{12a} containing more than 10 mcg. of the mixed vitamins per milligram of organic solids. Growth was promoted by two types of aureomycin fermentation products—a dry mixture of the mycelium plus a variable amount of filter-aid, and a concentrate of the whole fermented mash from which water had been removed by evaporation. Growth responses were also produced by crystalline aureomycin hydrochloride and by cultures of Streptomyces aureofaciens in which the aureomycin had been destroyed by alkaline hydrolysis. Responses were also obtained with saccharin-sulfathiazole, streptomycin and 3-nitro-4-hydroxyphenylarsonic

acid, but these substances appeared less potent than aureomycin. No responses were obtained with dried whey or dried brewer's yeast. The fact that growth responses have been obtained by such widely differing types of compounds supports the view that the growth produced by aureomycin is related to its antibiotic activity.

An addendum reports a manuscript shown the authors after submission of this article, in which Gyorgy Stokes, Smith and Goldblatt (*Tr. of Conf. on Biol. Antioxidants*, sponsored by the Macy Foundation, Nov. 1949; see also Abstr. 747) described a beneficial effect exerted by aureomycin on growth and prevention of dietary hepatic necrosis in rats. The assumption was made that aureomycin might act by suppressing the intestinal flora.

Laboratory of Microbiology
Pearl River, N. Y.

- 637 WHITEHILL, A. R., OLESON J. J., and HUTCHINGS, B. L. *Stimulatory effect of aureomycin on the growth of chicks*, Proc. Soc. Exper. Biol. & Med. 74: 11-13, May 1950.

On the basis of findings of other investigators that for chicks the growth-promoting activity of "animal protein factor" concentrates is not due to their B₁₂ content alone, and that Streptomyces aureofaciens fermentation liquors (of which aureomycin is a constituent) have increased growth, the growth-promoting effect of aureomycin, and certain other antibiotics were added to three test diets. All diets were supplemented with vitamin B₁₂ 90 mcg./kg.

Results indicate that the combined diet effected greater growth stimulation than that produced by vitamin B₁₂ alone. Of the other antibiotics given at the level of 100 mg./kg., only penicillin produced a response equivalent to that produced by aureomycin. The effect of aureomycin is presumed to be indirect, since certain other antibiotics manifest the same property.

Laboratory of Microbiology
Pearl River, N. Y.

638. OLESON J. J., HUTCHINGS, B. L., and WHITEHILL, A. R. *The effect of feeding aureomycin on the vitamin B₁₂ requirement of the chick*, Arch. Biochem. 29: 834-838, Dec. 1950.

The interdependence of the growth effects of vitamin B₁₂ and aureomycin in the chick were studied. Each factor appears to have a sparing action on the other. The effect of aureomycin on the growth of chicks fed no vitamin B₁₂ supplement and fed an excess of vitamin B₁₂ was determined. In the absence of vitamin B₁₂ no stimulation was obtained, while a definite growth increase was evident when vitamin B₁₂ was present. When chicks were fed sufficient levels of vitamin B₁₂ to give suboptimal and optimal growth responses, a marked weight increase was obtained by the addition of aureomycin.

An attempt was made to determine the minimal vitamin B₁₂ level essential to demonstrate the aureomycin growth effect; it appears that a level somewhere between 2.1 and 4.2 mcg. per Kg. of diet is required. From this level to 90 mcg. per Kg. of diet the antibiotic stimulatory effect was evident. Aureomycin gives growth stimulation at levels as low as 5 mg. per Kg. of diet.

Preliminary experiments with rats are described which show that several antibiotics (streptomycin, penicillin, streptomycin, and chloramphenicol) are capable of stimulating the growth of this species. The growth-stimulating effect of the antibiotics appears to be an indirect mechanism and not dependent upon the structure of the antibiotic.

Lohde Laboratories
Pearl River, N. Y.

639. BERG, L. R., BEARSE, G. E., MCGINNIS, J., and MILLER, V. L.: *The effect of removing supplemental aureomycin from the ration on the subsequent growth of chicks*, Arch. Biochem. 29: 404-407 Dec. 1950.

Chicks continued to gain weight when B_{12} was dropped from the diet at 4 weeks. Deletion of aureomycin from the ration resulted in a cessation of the accelerated rate of gain observed prior to the test.

640. COATES, M. E., HARRISON, G. F., KON, S. K., MANN, M. E., and ROSE, C. D.: *Effect of antibiotics and vitamin B_{12} on the growth of normal and animal protein factor deficient chicks* Biochem. J. 48: xii, Jan. 1951 (in Proc. Biochem. Soc.).

641. STOKSTAD, E. L. R., and JUDES, T. H.: *Effect of various levels of vitamin B_{12} upon growth response produced by aureomycin in chicks*, Proc. Soc. Exper. Biol. & Med. 76: 73-76, Jan. 1951.

Chicks on a vitamin B_{12} -deficient diet were fed graded levels of vitamin B_{12} in the presence and absence of streptomycin. In some experiments streptomycin seemed to have a sparing effect on vitamin B_{12} requirement, but in others no effect was observed. Aureomycin promoted growth both in the presence and absence of vitamin B_{12} supplements. The mortality of vitamin B_{12} -deficient chicks on the diet containing no vitamin B_{12} was reduced by streptomycin. The addition of sulfamethazine to the diet did not appear to affect the quantitative requirement of chicks for vitamin B_{12} .

This may be due to differences in the intestinal flora.

Lohde Laboratories
Pearl River, N. Y.

642. ATKINSON, R. L., and COUCH, J. R.: *Vitamin B_{12} in APP concentrate, streptomycin, streptomycin, liver "L" and fish meal and fish solubles in the nutrition of the poult*, J. Nutrition 44: 249-263, June 1951.

The addition of 66 mg. of streptomycin plus vitamin B_{12} 13 mcg. per Kg. of a corn-soybean oil meal ration containing bone meal, oyster shell, salt, manganese sulfate fortified fish oil, vitamin D and riboflavin provided for approximately 15 per cent increase in the growth rate of poults at 8 weeks of age. Similar increases were observed in birds receiving 2 per cent APP No. 4 (B_{12} 12 mcg./Kg.).

The injection of 2 mcg. of vitamin B_{12} per bird per week had no effect on growth and feed efficiency. The substitution of 4% liver "L" (B_{12} 49.4 mcg./Kg.) for an equivalent amount of soybean oil meal in the diet pro-

duced birds that weighed more than those fed 6% fish meal and 3% fish solubles. The liver "L" fraction also improved feed efficiency.

Mortality was higher in the groups receiving the B_{12} -streptomycin supplement (10%) or the fish meal-fish solubles (16%). A 5% mortality was observed in groups fed the APP concentrate, while only 2% mortality occurred in those given the B_{12} injections, liver "L" or the unsupplemented basal.

In a second trial, eight groups of 45 birds each were fed the same corn-soybean oil meal ration used in the first experiment plus the following added quantities of B-vitamins in milligrams per kg.: thiamine 4, riboflavin 6, calcium pantothenate 15, niacin 100, pyridoxine 4, folic acid 2, biotin 0.2, and choline 2,000. Significant increases in growth rate were obtained by supplementing this basal with Merck MK 23 (streptomycin 66 mg. plus B_{12} 13 mcg./Kg.) streptomycin 100 mg./Kg. alone or in combination with 2 mcg. of B_{12} injected per bird per week, 6% fish meal plus 3% fish solubles, or 4% liver "L" (B_{12} 49.6 mcg./Kg.). The greatest growth response of approximately 11% was obtained on the streptomycin B_{12} fortified diet. Birds on the basal alone weighed more than did those injected with 2 mcg. of B_{12} per week.

Poults on the streptomycin B_{12} , liver "L" and B_{12} (injected) supplemented diets were continued on test for an additional six weeks to determine whether the weight increases which were apparent at eight weeks would continue to persist. The streptomycin- B_{12} and injected B_{12} continued to accelerate the growth of the turkey poults up to and including the fourteenth week of the growth period.

When a comparison was made between the weights of birds on the corn-soybean basal and those on the B-vitamin fortified basal similarly supplemented, it was found that the 8 crystalline B-vitamins promoted the growth of poults in some instances but failed to have an appreciable effect in the presence of streptomycin and B_{12} or when 2% APP (B_{12} 12 mcg./Kg.) supplements were fed. Feed efficiency and mortality data were also given for the second trial.

Several possibilities were discussed as to the mode of action of the antibiotics in stimulating poult growth.

Agricultural and Mechanical College of Texas
College Station, Texas

643. ELAM, J. E., GEE, L. L., and COUCH, J. R.: *Function and metabolic significance of penicillin and bacitracin in the chick*, Proc. Soc. Exper. Biol. & Med. 78: 832-836, Dec. 1951.

The effect of oral and parenteral administration of penicillin, bacitracin, and autoclaved penicillin on growth and aerobic fecal microflora of chicks was studied. The New Hampshire chicks used in these trials were obtained from hens fed a corn-milo-soybean oil meal basal ration fortified with fish meal, an APP- B_{12} concentrate, vitamins and minerals. Sixteen groups of 50 chicks each were used and were kept in batteries with raised screen floors for a 10-week experimental period.

The administration of oral or injected penicillin (amounts employed not given) increased the rate

growth. A slightly greater growth increase was obtained when penicillin in sesame oil (containing aluminum monostearate) and autoclaved penicillin were each injected.

The injection of vitamin B₁₂ resulted in an increase in growth. When both B₁₂ and penicillin were given an additional growth response was produced.

Bacitracin given orally or parenterally increased growth over that obtained on the unsupplemented basal; this increase was of the same magnitude as that produced by adding B₁₂ alone to the diet. Birds receiving bacitracin orally or parenterally with B₁₂ weighed more at the end of the 10-week test period than did those of any other group.

The oral administration of penicillin produced an increase in the penicillin and aureomycin-resistant organisms and in yeast counts. There was no significant increase in the total dilution count, enterococci, lactics, or coliforms in the feces of chicks receiving oral penicillin or the unsupplemented basal. Penicillin given parenterally had no effect on the aerobic fecal microflora. When penicillin was administered in sesame oil containing aluminum monostearate, there was an increase in the penicillin-resistant organisms and in the yeast counts.

Bacitracin given orally or parenterally with or without B₁₂ had no effect on the thioglycollate count, enterococci, lactics, yeasts, coliforms, or penicillin or bacitracin-resistant organisms.

The results obtained in these experiments lead the authors to believe that the antibiotic molecule or a fragment thereof might act as a metabolite within the body of the bird.

*Agricultural and Mechanical College of Texas
College Station, Texas*

- 644 LIBBY D., and MEITES J. *Effects of vitamin B₁₂ and penicillin on thioauracil action in chicks*, Proc. Soc. Exper. Biol. & Med. 79 370-372, March 1952.

The effect of vitamin B₁₂ and penicillin, both of which have been shown to stimulate chick growth, was studied in a total of 168 thioauracil-treated chicks.

Authors summary "1. White Leghorn male and Rhode Island Red chicks of both sexes were fed a vit. B₁₂-deficient ration which was supplemented with 0.1% thioauracil and/or 0.5 to 4.0 µg. % vit. B₁₂ or 2 mg. procaine penicillin G per lb. of food. After a preliminary depletion period of one and 2 weeks, respectively on the vit. B₁₂-deficient diet, the chicks were fed the above supplements for an additional 4 or 5 week period. 2. Thioauracil markedly reduced body weight gains and food intake in both breeds of chicks, and increased the grams of food required for each gram of gain in body weight. Vit. B₁₂ and penicillin partially or completely counteracted this growth depression and increased appetite. The larger doses of vit. B₁₂ were more effective in these respects than the smaller doses. 3. The large increase in thyroid weight and severe inhibition of comb growth induced by thioauracil were not influenced by the vitamin or antibiotic. 4. In general, these results corroborate previous data on the effects of vit. B₁₂ and

antibiotics on rats fed thioauracil or following parathyroidectomy"

*Washington State College
East Linn, Wash.*

Rats

- 645 STERN J R., and MCGINNIS J. *Antibiotics and early growth of rats fed a soybean oil meal diet*, Arch. Biochem. 28 364-370 Oct. 1950.

The diet used in this study contained soybean oil meal as protein and glucose as carbohydrate. Growth with B₁₂ plus antibiotics aureomycin, terramycin or streptomycin was better than with vitamin B₁₂ alone.

"It is likely that the increase in growth rate is mediated through alterations in the intestinal flora of the rat, and that the carbohydrate source as well as the antibiotic, is capable of changing the bacterial population of the digestive tract."

- 646 CRAVIOTO-MUNOZ, J., PONCHER, H. G., and WAISMAN H. A. *Vitamin B₁₂ sparing action of aureomycin in the rat*, Proc. Soc. Exper. Biol. & Med. 77 18-19 May 1951.

Aureomycin included in the purified diet of rats at levels of 5 and 10 mg. per 100 Gm. gives growth increases equal to or better than those obtained with vitamin B₁₂ at a level of 0.01 mg. per 100 Gm. This may be explained by the suppression, in the intestine, of bacteria which utilize vitamin B₁₂ for their own growth and make it unavailable to the rat, or it may be that the suppression of some microorganisms allows others which synthesize vitamin B₁₂ to increase in number

*University of Illinois College of Medicine
Chicago, Ill.*

- 647 MEITES J., and OGLE, R. C. *Antithyrotic effects of antibiotics in rats*, Proc. Soc. Exper. Biol. & Med. 77 758-761 Aug. 1951.

Authors summary "(1) Three crystalline antibiotics were fed to 100 male and female rats to determine whether they could counteract the retardation of growth induced by feeding thyrotoxic amounts of Protamone. On a ration adequate in vitamin B₁₂, penicillin or neomycin but not streptomycin were completely effective in overcoming the growth-inhibition, while on a ration deficient in vitamin B₁₂, all 3 antibiotics were only partially effective. On the deficient ration, the antibiotics reduced the survival rate of the hyperthyroid rats. (2) At least part, if not all, of the effectiveness of the antibiotics in counteracting growth retardation could be attributed to the increase in food consumption and to an increased efficiency in converting food into body weight gains. The antibiotics did not alter the 74% increase in basal oxygen consumption induced by the Protamone, nor did they change the action of the latter on adrenal and spleen weight. Thyroid atrophy was partially or completely prevented by the antibiotics."

*Washington State College
East Linn, Wash.*

648. MEITES J. *Counteraction of cortisone inhibition of body hair and thymus growth by vitamin B₁₂ and aureomycin*, Proc. Soc. Exper. Biol. & Med. 78 692-695 Dec. 1951.

Inhibition of body hair and thymus growth induced in vitamin B₁₂-deficient rats by the subcutaneous injection of 0.5 mg. of cortisone daily was completely or partially prevented by incorporating 200 mcg. of vitamin B₁₂ per g. of ration or 0.005 per cent of aureomycin. Vitamin B₁₂ was more effective than aureomycin, and the combination of both was more effective than either alone. Increase in food consumption and greater efficiency in converting food into gains in body weight was present during vitamin and antibiotic treatment. The author concludes that large doses of cortisone aggravate existing vitamin B₁₂ deficiency and that this accounts, at least partially for inhibition of body and hair growth. Experiments are under way which indicate that large doses of cortisone (1 mg. daily) may induce deficiencies of vitamin B₁₂ even when a diet adequate in this vitamin is given. The action of vitamin B₁₂ and aureomycin upon the thymus appears to be direct, but its mechanism is still unknown.

An addendum mentions the appearance, while this paper was in press, of an article by Wahlstrom and Johnson (*Proc. Soc. Exper. Biol. & Med.* 78 112, 1951 [Abstr. 655]) which appears to confirm the theory that large doses of cortisone increase requirements for vitamin B₁₂.

*Michigan State College
East Lansing, Mich.*

- 649 MEITES, J. Beneficial effects of vitamin B₁₂ and aureomycin in rats given large doses of cortisone. *Proc. Soc. Exper. Biol. & Med.* 81 307-311 Oct. 1952.

Author's summary: "1. Fifty young male albino rats were uniformly divided into 5 equal groups and placed on a purified casein-glucose diet. After a preliminary period of 2 weeks, all except a control group were injected for 28 days with 1 mg. of cortisone acetate daily with or without vitamin B₁₂ and/or aureomycin. 2. Cortisone acetate alone induced complete inhibition of body growth, severe alopecia and marked atrophy of the thymus gland. Vitamin B₁₂ (200 µg./kilo diet) or aureomycin (0.005%) largely counteracted these inhibitory effects of cortisone acetate, and the combination of the two was more effective than either alone. It was concluded that large doses of cortisone acetate given to young rats on a casein-glucose diet, increased their requirements for vitamin B₁₂ and other factors. These data corroborate results previously obtained in a similar experiment on rats fed a corn-soybean meal diet."

*Michigan State College
East Lansing, Mich.*

Swine

- 650 CARPENTER, L. E. Effect of aureomycin on the growth of weaned pigs. *Arch. Biochem.* 27 469-471, July 1950.

Pigs fed a diet of natural feedstuffs including animal by-products may respond to additional dietary vitamin B₁₂.

- 651 SPEER, V. C., VOHS, R. L., CATRON D. V., MADDOCK, H. M., and CULBERTSON C. C. Effect of aureomycin and animal protein factor on healthy pigs. *Arch. Biochem.* 29 452-453, Dec. 1950.

The improvement of pig gains by the addition of APF may be explained by the contribution of the APF preparations used of additional vitamin B₁₂ and/or their content of unidentified growth factors.

- 652 CUNHA, T. J., MEADOWS G. B., EDWARDS H. M., SEWELL, R. F., PEARSON A. M., and GLASSCOCK, R. S. A comparison of aureomycin, streptomycin, penicillin and aureomycin-B₁₂ feed supplement for the pig. *Arch. Biochem.* 30 260-271 Feb. 1951.

A feed supplement containing penicillin and vitamin B₁₂ had no beneficial effect on the growth of pigs. A supplement containing vitamin B₁₂ and streptomycin stimulated growth to a small extent. Supplementation of the diet with vitamin B₁₂ and aureomycin produced a considerable increase in growth of the pigs.

*Florida Agricultural Experiment Station
Gainesville, Fla.*

- 653 CARPENTER, L. E. The effect of antibiotics and vitamin B₁₂ on the growth of swine. *Arch. Biochem. & Biophysics* 32 187-191, June 1951.

Aureomycin, terramycin, penicillin, streptomycin, and chloromycetin stimulated the growth of pigs at the level tested, under the environmental conditions imposed. All the antibiotics except chloromycetin controlled diarrhea. Vitamin B₁₂ and streptomycin given alone, either orally or parenterally stimulated the growth of weaned pigs. No greater effects were obtained when both were given together. The vitamin had no effect on the incidence of diarrhea, but streptomycin administered either orally or parenterally controlled diarrhea.

- 654 CATRON D. V., MADDOCK, H. M., SPEER, V. C., and VOHS, R. L. Effect of different levels of aureomycin with and without vitamin B₁₂ on growth of weaned swine. *Antibiotics & Chemother.* 1 81-84, April 1951.

Aureomycin hydrochloride significantly increased weight gain and feed efficiency. Crystalline vitamin B₁₂ increased weight gain but not feed efficiency. There was no complementary effect of aureomycin and vitamin B₁₂.

- 655 WAHLSTROM, R. C., and JOHNSON B. C. Effect of cortisone and of aureomycin on baby pigs fed a vitamin B₁₂ deficient diet. *Proc. Soc. Exper. Biol. & Med.* 78 112-114, Oct. 1951.

Aureomycin did not alleviate and cortisone enhanced the experimental vitamin B₁₂ deficiency in baby pigs.

Dogs

- 656 ARNRICH, L., LEWIS, E. M., and MORGAN A. F. Growth of dogs on purified diet plus aureomycin and/or Vit. B₁₂. *Proc. Soc. Exper. Biol. & Med.* 80 401-404, July 1952.

Authors' summary: "Eleven young cocker spaniels from 2 litters were fed from weaning for 20 weeks a purified complete diet which had been shown previously produce optimum growth. Vit. B₁₂ at the level of 5 aureomycin hydrochloride at the level of 10

both these substances were added to the diet of some of the animals. No differences resulted in nitrogen retention or in the nitrogenous constituents of the blood of any of the dogs. Two of the 3 dogs which received vit. B₁₂, 3 of the 4 which received both vit. B₁₂ and aureomycin and one of the 2 which received only aureomycin gained more weight per 100 g. food than did the 2 controls and the 3 others. These overgrown dogs had greater total gains, greater empty carcass weights, lower body specific grav-

ity more carcass fat, and more fat-rich adipose tissue than the 5 others. However the fat free carcasses were not significantly heavier. It is concluded that better food utilization may result in dogs in some cases from the addition of vit. B₁₂ and/or aureomycin to a complete purified diet and that this growth is due solely to increased fat deposition."

University of California
Berkeley, Calif.

METABOLISM

AMINO ACIDS; VITAMINS; RELATED COMPONENTS

Chicks

- 657 RICHARDSON, L. R., BLAYLOCK, L. G., GERMAN, H. L., and SHERWOOD, R. M. *Amino acids and vitamin B₁₂ as supplements to plant proteins for growing chicks*, Federation Proc. 8 393, March 1949

Chicks on a cottonseed meal diet supplemented with lysine weighed more than those on the diet without the amino acid. Tryptophane and methionine increased the weight still more.

A vitamin B₁₂ concentrate added to a cereale-cottonseed meal diet supplemented with lysine, tryptophane and methionine increased the weight of chicks. Three chicks on the diet without vitamin B₁₂ weighed, on the average, 228 Gm., while 3 on the diet with 40 mcg. vitamin B₁₂ per Kg. averaged 300 Gm. Two groups of 17 chicks each on the cereale-soybean meal diet averaged 250 Gm. without and 285 Gm. with vitamin B₁₂.

658. SCOTT, M. L., HILL, C. H., and NORRIS, L. C. *Factors concerned in the liberation of pteroylglutamate (folic) acid from its heptaglutamate by chick liver* Federation Proc. 8 249 March 1949

Experiments are reported which indicate that vitamin B₁₂ is required in addition to p-pyrazin in the enzymatic release of folic acid from pteroylglutamate. Both vitamin B₁₂ concentrates and crystalline vitamin B₁₂ have been added to the liver incubation system with and without p-pyrazin, in these experiments.

- 659 GILLIS, M. B., and NORRIS, L. C.: *Effect of the animal protein factor on the requirements for methylating compounds*, J. Biol. Chem. 179 487-488, May 1949 (Letter to Editors)

In experiments with chicks, a diet complete in all known vitamins and composed of mixed cereals, plant protein, and supplementary minerals and vitamins was significantly improved by additions of betaine or choline. Since betaine was as effective as choline in promoting the growth of chicks on this diet, the effect was probably due to the increased methyl groups provided. However the addition of 0.15 per cent of liver extract paste was considerably more effective than betaine or choline in promoting growth. No further improvement was obtained by adding betaine or choline to the diet when liver paste was included. The amount of choline contributed by the liver paste was negligible. The liver paste used is a source

of animal protein factor (APF) and contains approximately 17 micrograms of vitamin B₁₂ activity per gram by assay with *Lactobacillus leichmannii*. The results obtained in these studies show that the inclusion of a source of APF relieved the need for supplementary methylating compounds, indicating that at least one metabolic function of APF is concerned with transmethylation. It is probable either that transmethylation is more efficient in the presence of an adequate amount of APF or that a partial deficiency of APF creates or stimulates metabolic processes which require excess methyl groups. Further work is required to show whether the activity of the liver paste is due to vitamin B₁₂ or to some other component of APF

Cornell University
Ithaca, N. Y.

660. SCHAEFER, A. E., SALMON, W. D., and STRENGTH, D. R.: *Interrelationships of vitamins B₁₂ and choline. II. Effect on growth of the chick*, Proc. Soc. Exper. Biol. & Med. 71 202-204, June 1949

The choline requirement of chicks on a choline-low basal diet supplemented with choline was significantly reduced by giving supplements of vitamin B₁₂. Crystalline vitamin B₁₂ fed at a level of 15 mcg. per Kg. of diet was as effective as vitamin B₁₂ concentrate in supplementing a diet at a choline level of 0.1 per cent over a two-week feeding period. These findings indicate that dietary choline has a significant sparing action on vitamin B₁₂.

- 661 WILLIAMS, J. N., NICHOL, C. A., and ELVEHJEM, C. A. *Relation of dietary folic acid and vitamin B₁₂ to enzyme activity in the chick*, J. Biol. Chem. 180 689-694, Sept. 1949

Chicks fed a diet supplemented with vitamin B₁₂ without folic acid show higher p-amino acid oxidase activity than those not receiving vitamin B₁₂.

662. CHARKEY, L. W., WILGUS, H. S., PATTON, A. R., and GASSNER, F. X. *Vitamin B-12 is amino acid metabolism*, Proc. Soc. Exper. Biol. & Med. 73 21-24, Jan. 1950.

In chicks on a basal diet supplemented with the equivalent of 50 mcg. of vitamin B₁₂ per Kg. of feed a reduction occurred, as compared with control chicks, in the blood levels of nonprotein nitrogen and of seven individual amino acids (arginine, lysine, methionine, tryptophane, histidine, threonine, valine). The chicks given vitamin B₁₂ grew more rapidly and utilized feed more efficiently than the B₁₂-deficient controls, although the

latter had higher blood levels of amino acids. Vitamin B₁₂ appears to function in metabolism by enhancing utilization of circulating amino acids for building fixed tissues.

*Colorado Agricultural Experiment Station
Fort Collins, Colo.*

663. JUKES, T. H., STOKSTAD E. L. R., and BROQUIST H. P.: *Effect of vitamin B₁₂ on the response to homocystine in chicks*, Arch. Biochem. 25: 453, Feb. 1950 (in Letters to the Editors)

"Homocystine, with or without betaine, did not promote the growth of vitamin-B₁₂-deficient chicks on a diet deficient in methionine. However these chicks responded to the addition of methionine. When supplemented with B₁₂, the chicks responded to methionine, homocystine, or homocystine plus betaine.

"Vitamin B₁₂ may be needed for the transamination of homocystine to methionine in chicks. Other functions for vitamin B₁₂ in the nutrition of chicks are indicated by the observation that growth was more rapid on methionine plus B₁₂ than on methionine alone, and by the finding that vitamin B₁₂ is needed for growth and survival of chicks on a diet of natural foods supplemented with methionine and choline."

*Laboratory Laboratories
Pearl River, N. Y.*

664. MACHLIN, L. J., MILLIGAN, J. L., DENTON C. A., and BIRD H. R.: *Effect of various amino acids and vitamin B₁₂ on chick growth*, Federation Proc. 9: 364 March 1950.

665. GILLIS M. B., and NORRIS L. C.: *The effect of vitamin B₁₂ on the response of chicks to betaine and choline*, J. Nutrition 43: 295-302, Feb. 1951

Authors summary "Under the experimental conditions reported above the addition of betaine or choline to a practical ration based on cereals and vegetable protein resulted in a significant improvement of the ration as measured by chick growth. In several experiments with White Leghorn chicks having normal body stores of vitamin B₁₂, the growth responses due to either betaine or choline supplements were approximately equal. Chicks having subnormal reserves of vitamin B₁₂, however, utilized betaine more effectively than choline. In 5 experiments with vitamin B₁₂-depleted chicks a significantly greater gain in weight resulted from adding betaine to the basal diet than from adding choline. The evidence suggests that at least one mechanism by which the chick utilized choline is impaired by a deficiency of vitamin B₁₂, while betaine retains its effectiveness under the same conditions."

*Cornell University
Ithaca, N. Y.*

666. JUKES, T. H., and STOKSTAD E. L. R.: *Studies of vitamin B₁₂, choline and related factors in the diets of chicks* J. Nutrition 43: 459-467 April 1951.

Authors summary "Choline deficiency as produced by a purified diet was studied in chicks which were also deficient in vitamin B₁₂. The requirement of choline for

maximum growth was found to be greater in the absence of vitamin B₁₂ than in its presence. However the amount of choline required for the prevention of perosis was not decreased by supplying vitamin B₁₂. Betaine did not have a marked effect when added to diets containing an insufficient level of choline. A methionine-deficient diet was fed to vitamin B₁₂-deficient chicks and no evidence of a sparing effect of vitamin B₁₂ on the methionine requirement was noted under the experimental conditions encountered. Choline deficiency was not encountered on a diet of natural foods containing a high level of soybean meal."

*Laboratory Laboratories
Pearl River, N. Y.*

667. GILLIS, M. B., and NORRIS L. C.: *Methylation of homocystine by chicks deficient in vitamin B₁₂*, Proc. Soc. Exper. Biol. & Med. 77: 18-15, May 1951.

Chicks severely depleted of their vitamin B₁₂ reserves retain the ability to perform the overall reaction homocystine plus labile methyl forms methionine. If vitamin B₁₂ is needed for the completion of this reaction, the amount required must be exceedingly small. It seems probable, however that vitamin B₁₂ is concerned in the synthesis of methyl groups or the utilization of such synthesized groups by the chick.

*Cornell University
Ithaca, N. Y.*

668. SCHAEFER, A. E., SALMON W. D., and STRENGTH, D. R.: *The influence of vitamin B₁₂ on the utilization of choline precursors by the chick*, J. Nutrition 44: 805-811, June 1951.

Data are presented which indicate that vitamin B₁₂ is involved in the utilization of choline precursors for choline synthesis.

669. JUKES, T. H.: *Factors involved in utilization of homocystine by chicks* Federation Proc. 11: 447 March 1952.

"Vitamin B₁₂-depleted chicks were fed diets deficient in methionine, choline and vitamin B₁₂. The response to homocystine was markedly increased by vitamin B₁₂ under various dietary conditions. However in the absence of vitamin B₁₂ homocystine often depressed growth. The presence or absence of vitamin B₁₂ or folic acid had little or no effect on the response to methionine. Choline appeared to be highly effective as a methylating agent for homocystine in the presence of vitamin B₁₂ for chicks on a diet markedly deficient in methionine. The response to vitamin B₁₂ was greater in the presence of folic acid than in its absence and was greater in the presence of homocystine than in its absence when methionine was omitted. The response to folic acid was usually diminished by adding choline. The addition of homocystine and vitamin B₁₂, or homocystine and choline to the basal diet gave poor growth, but excellent growth was obtained when homocystine, vitamin B₁₂ and choline were all three added. With vitamin B₁₂ added, betaine was much less effective than choline in improving the response to homocystine unless dimethylaminoethanol was added, in which case betaine was almost as effective as choline. S formate or additional glycine had little effect

utilization of homocysteine for growth in the presence of vitamin B₁₂. No response to methionine was obtained in chicks deficient in both pyridoxine and methionine. The requirement for pantothenate was not significantly affected by vitamin B₁₂-deficiency in chicks on a diet adequate in methionine and choline."

Ludwig Laboratories
Purdue Univ. W. I.

670. SCHWEIGERT B. S. *Amino acids and vitamins*, Chem. & Engin. News 30 1289 March 31, 1952 (In Soc. Proc.)

"Reaction mechanisms involved in the conversion of tryptophan to niacin were outlined. The production of quinolinic acid from the intermediate compound, 3-hydroxyanthranilic acid, is unique in that this involves the conversion of a benzene-type compound to a pyridine-type compound."

The speaker "suggested that riboflavin and vitamin B₆ function in a catalytic manner. In response to a question he indicated that a growth rate reduced because of high levels of glycine might be restored by the addition of vitamin B₁₂ to the diet. [He] pointed out that the presence of B₁₂ has been shown to reduce the choline requirement necessary for chick growth."

"The conversion of tryptophan to niacin is the best example of an amino acid as a direct precursor to a vitamin."

American Heart Institute Foundation
Chicago, Ill.

Rats

671. SCHAEFER, A. E., SALMON, W. D., and STRENGTH, D. R. *Relation of vitamin B₁₂ to choline requirement of the rat and chick*, Federation Proc. 8 395, March 1949

The severity and incidence of renal hemorrhage in weanling rats on diets deficient in choline and substitute nutrients was decreased by supplementing the diet with a vitamin B₁₂ concentrate. The incidence of renal hemorrhage within 14 days in chicks on a diet supplemented with 0.04% of choline was 100% and on a diet supplemented with 0.06% was 66%. The addition of a concentrate supplying 8 mcg. of vitamin B₁₂ per 100 Gm. of diet to diets supplemented with 0.03, 0.04, and 0.06% of choline reduced the incidence of renal damage to 50%, 36% and 0, respectively. Preliminary results indicated that on the basis of methyl equivalents DL-methionine had 100% of the theoretical potency of choline chloride when vitamin B₁₂ was added to the diet. A diet which required a supplement of 0.6% of choline to induce maximum growth in the absence of vitamin B₁₂ resulted in an increased growth increment of 75 Gm. in six weeks when supplemented with 8 mcg. of vitamin B₁₂ per 100 Gm. At eight weeks the weight gain of chicks receiving only 0.2% of choline plus vitamin B₁₂ was equal to that of chicks receiving 0.6% of choline without vitamin B₁₂.

672. SCHAEFER, A. E., SALMON, W. D., and STRENGTH, D. R. *Interrelationship of vitamin B₁₂ and choline. I Effect on hemorrhagic kidney syndrome in the rat*, Proc. Soc. Exper. Biol. & Med. 71 193-196, June 1949

Incidence and severity of renal injury in weanling rats fed diets low in choline and methionine were significantly decreased by supplementing the diet with a vitamin B₁₂ concentrate or crystalline vitamin B₁₂. Under the conditions of these experiments, 80 mcg. vitamin B₁₂ per Kg. of diet could replace about half of the supplementary choline or methionine required for protection against kidney damage. When rats on subprotective levels of choline were given vitamin B₁₂ in addition, their weight increased significantly but the addition of vitamin B₁₂ to an adequate level of choline did not produce an increase in weight gain. These findings establish the existence of an interrelationship between vitamin B₁₂ and choline or methionine.

Glaxo Pharmaceutical Institute
Nutley, N. J.

673. SCHULTZ, M. O. *Nutritional value of plant materials. II Prevention of acute uremia of the newborn rat by vitamin B₁₂*, Proc. Soc. Exper. Biol. & Med. 72 613-616, Dec. 1949

Author's summary: "Acute uremia of the newborn rat observed on rations in which a commercial soybean protein and DL-methionine furnish the only source of amino acids can be prevented by subcutaneous administration of 0.05 µg. of vitamin B₁₂ shortly after birth."

University of Minnesota
St. Paul, Minn.

674. SCHAEFER, A. E., SALMON, W. D., STRENGTH, D. R., and COPELAND, D. H. *Interrelationship of folacin, vitamin B₁₂, and choline. Effect of hemorrhagic kidney syndrome in the rat and on growth of the chick*, J. Nutrition 40 95-112, Jan. 1950 (abstr. J. Am. Dietet. A. 26 450, June 1950)

"The incidence and severity of renal injury in weanling rats fed diets low in choline and methionine were decreased by supplementing the diet with folacin. When both folacin and vitamin B₁₂ were added to the basal diet supplemented with 0.04 per cent choline chloride or 0.128 per cent DL-methionine, there was complete protection against kidney damage. Evidence is offered that the nutritional requirements of the chick and rat for folacin, vitamin B₁₂, and choline are interrelated, and a specific requirement for one of these nutrients cannot be established unless the level of the other two is taken into consideration."

675. STEKOL, J. A., BENNETT, M. A., WEISS, I., HALPERN, P., and WEISS, S. *Sulfur-containing amino acids in growth with a 'labile methyl' free diet containing vitamin B₁₂*, Federation Proc. 9 234 March 1950.

"We reported previously that 30 days or older rats are able to grow on a diet which was free of all the known vitamin B₁₂ methyl group donors but which contained vitamin B₁₂ and homocysteine (Abstracts, Am. Chem. Soc., 116th meeting, Sept. 18, 1949 55C). The amino acid diet which was used in the above studies was complete with respect to all the essential amino acids, except for methionine, and it contained all the known vitamins, including folic acid and biotin. We now find that on this diet, in the presence of vitamin B₁₂, homocysteine produces even better growth response than equivalent amounts of homo-

cystine. Substitution of homocysteine by L- and L-allo-cystathionine resulted in cessation of growth, which was resumed as soon as the cystathionines were replaced by homocysteine. On a diet containing ample methionine as the sole sulfur-amino acid, but which was free of vitamin B₁₂, poor growth was obtained supplementing the methionine-containing diet with vitamin B₁₂ resulted in good growth. None of the experimental animals receiving vitamin B₁₂ died of kidney lesions in spite of the complete absence of labile methyl group donors in the diet."

Lambert Hospital Research Institute and
Institute for Cancer Research
Philadelphia, Pa.

676. DUBNOFF J W *Effect of vitamin B₁₂ on the reduction of S-S compounds in vitro* Federation Proc. 9 166, March 1950.

"Addition of vitamin B₁₂ concentrates to liver slices and homogenates increases the reduction of S-S compounds by preformed and added hydrogen donors. The specificity of hydrogen donors and the relation of this effect to methionine synthesis from homocysteine will be discussed."

California Institute of Technology
Pasadena, Calif.

677. SALMON W D: *Effects of protein level, vitamin B₁₂, and folic acid on utilization of methionine for transmethylation*, Federation Proc. 9 369 March 1950.

"Weanling rats were fed choline-free diets containing 7 or 9% of casein and corn gluten meal and sufficient DL-methionine to prevent kidney lesions in a 2-week period. When 10% of gelatine was added, at the expense of sucrose no kidney damage occurred; the further addition of 0.10% DL-tryptophan resulted in 100% incidence of renal damage but tryptophan without gelatine had no effect. Vitamin B₁₂ alone did not decrease incidence of kidney damage by the gelatine-tryptophan-containing diet but vitamin B₁₂ and folic acid together decreased incidence 50%. A 50-100% incidence of renal injury developed in rats receiving a choline-free, synthetic diet containing 60% of alcohol extracted casein. Vitamin B₁₂ and folic acid, or these vitamins together with 0.64% DL-methionine, did not consistently increase growth or decrease incidence of renal damage. Choline alone (0.20%) prevented kidney lesions but had little effect on growth. Choline together with vitamin B₁₂ and folic acid produced normal growth and grossly normal kidney condition; ethanolamine appeared to be as effective as choline in this combination. The results indicate a significant effect of protein level on utilization of methionine and a specific relationship of vitamin B₁₂ and folic acid to transmethylation in the rat."

678. SIMMONDS, S., KELER, E. B., CHANDLER, J P., and DE VIGNEAUD V *The effect of ethionine on transmethylation from methionine to choline and creatine in vivo* J Biol. Chem. 183 191 195, March 1950

Experiments with rats showed that ethionine decreased the amount of transmethylation from methionine to choline by about 20 per cent.

Cornell University Medical College
New York, N Y

679. SCHULTZE, M O *Nutritional value of plant materials. I Growth of rats on purified rations containing soybean protein*, J Nutrition 41 103-113, May 1950.

Author's summary "Rats fed purified rations containing 24% protein in the form of a soybean protein preparation and DL-methionine as the only source of amino acids made satisfactory post weaning weight gains for 4 successive generations. Extensive purification of the soybean protein did not decrease the growth rate of rats consuming this protein as compared to that of animals consuming a less purified preparation. Daily administration of 0.25 µg. of crystalline vitamin B₁₂ to rats on a ration containing a highly purified soybean protein preparation did not increase the early post-weaning weight gains or the efficiency of food utilization."

University of Minnesota
St. Paul, Minn.

680. DUBNOFF J W *The effect of B₁₂ concentrates on the reduction of S-S groups*, Arch. Biochem. 27 466-467 July 1950.

The influence of vitamin B₁₂ on transmethylation reactions was studied. The results suggest a B₁₂ component acting as a hydrogen carrier to S-S groups. This may explain the effect of B₁₂ on methionine formation and the fact that B₁₂ may be at least partially replaced as a microbiological growth factor by reducing agents or by lowering the oxygen tension of the medium. Other apparently diverse *in vitro* activities of B₁₂ which have been reported may be the result of the influence of B₁₂ on glutathione and S-H groups which in turn can activate a wide variety of enzyme systems.

681. DINNING J S., PAYNE, L. D., and DAY P L. *The requirements of rats for methyl groups and vitamin B₁₂ in the production of leukocytes* Arch. Biochem. 27 467-469 July 1950 (Letter to Editors)

Results of a study of the requirement of labile methyl for leukocyte formation and of the effect of vitamin B₁₂ in the utilization of methyl groups suggest that B₁₂ may be essential for the utilization of methyl groups of betaine.

682. WEISSBACH, A., ELWYN D., and SPRINSON D B. *The synthesis of the methyl groups and ethanolamine moiety of choline from serine and glycine in the rat*, J Am. Chem. Soc. 72 3316-3317 July 1950 (In Communications to the Editor)

Experiments are reported which show that the rat can synthesize methyl groups from serine, or from glycine converted to serine. This is in agreement with the recent report (Bennett, *Science* 110: 589 1949) that diets devoid of methyl group donors will support growth when folic acid and vitamin B₁₂ are present.

Columbia University College of Physicians and Surgeons
New York, N Y

683. CARTER, R. E., BUSCH, E., and STRANG, V *The effect of vitamin B₁₂ on the leukopenia induced by radiation*, Blood 5 753-757 Aug. 1950.

Observation that thymidine can replace B_{12} as a growth factor for lactobacilli had led to the postulation that B_{12} functions as a coenzyme in the conversion of thymine to thymidine. To determine a possible interference with nucleoprotein formation produced by radiation, on the basis of its known effects on living tissue, a trial of the effects of vitamin B_{12} on radiation-induced leukopenia in rats was made.

Authors' conclusions: "Crystalline vitamin B_{12} administered via intramuscular injection, in single and in multiple doses, produced no effect on the leukopenia induced in rats by 400 roentgens of 250 kv x-rays."

Los Alamos Scientific Laboratory
University of California
Los Alamos, N. M.

- 684 DUVIGNEAUD V., RESSLER, C., and RACHELE, J. R. The biological synthesis of "labile methyl groups." *Science* 112 267-271, Sept. 8, 1950.

This paper is a résumé of the present status of the concept of the synthesis of biologically labile methyl groups. The authors first review a number of studies made by other workers on the growth of rats maintained on a methionine-free, homocystine-containing diet supplemented with choline, with crude liver extract, or with vitamin B_{12} alone or together with folic acid. It appeared likely that vitamin B_{12} and folic acid may be involved in the synthesis of labile methyl groups.

The authors then report the results of investigations with germ free rats (conducted in collaboration with James A. Reyniers, Thomas D. Luckey et al. at LOREY of the University of Notre Dame) the purpose of which was to obtain evidence as to where the labile methyl groups are synthesized in the thorax, or in the intestines, mediated through bacterial action. The technique involved the detection of the synthesis of methyl groups through the isolation of choline from rats, the body water of which contained deuterium oxide (D_2O). Rats of the LOREY strain were maintained with D_2O in their drinking water under both germ-free and nonsterile conditions at LOREY. For purposes of comparison, animals of the Rockland Farms strain were kept at the Cornell Laboratories in New York under ordinary laboratory conditions on the same dietary regimen as that used at LOREY. All determinations were made in New York, the LOREY sterile specimens being shipped there by air express. All animals received the LOREY diet (identified in detail) one pair of rats received additional supplements of vitamin B_{12} . It was found that deuterium was present in significant amounts in the methyl groups of choline isolated from the germ-free animals; this means that these animals were able to synthesize methyl groups. In the LOREY animals maintained under nonsterile conditions a higher value (which may not be significant) was obtained. The authors believe that, in these animals, additional synthesis of labile methyl groups may have been mediated through intestinal bacteria. The diets enriched with additional vitamin B_{12} did not increase the degree of methyl synthesis. It is concluded that biologically labile methyl groups can be synthesized by the tissues of the rat.

The authors then interpret the results of other workers in the light of their findings. They believe that, although labile methyl groups can be synthesized by the

animal, the rate of synthesis is not fast enough for the demands of the growing young rat. Biologically labile methyl groups can, therefore, be regarded as an essential dietary component.

Thirty-three references are cited.

Cornell University Medical College
New York, N. Y.

- 685 STEKOL, J. A., and WEISS K.: Vitamin B_{12} and growth of rats on diets free of methionine and choline, *J. Biol. Chem.* 185 343-350, Sept. 1950.

Rats aged 80 days or more maintained on diets free of labile methyl groups and containing homocystine but no vitamin B_{12} continued growing for two or three weeks and then lost weight and died with severely hemorrhagic kidneys. If vitamin B_{12} was administered during the period of loss of weight the animals immediately resumed growth. All rats continued growing on such diets if vitamin B_{12} was included from the start. The initial growth of rats on vitamin B_{12} -free diets is probably a function of the B_{12} stored in the liver. Replacing a portion of homocystine by cystine, or replacing homocystine by homocystine resulted in better growth rates. Replacing the homocystine by a mixture of L-cystathionine and L-allo-cystathionine resulted in loss of weight in spite of the presence of vitamin B_{12} . If choline was added in the cystathionine diet, slow growth was obtained.

Rats aged 22 to 24 days did not as a rule survive on the labile methyl free diet in spite of the presence of vitamin B_{12} , and usually developed damaged kidneys.

The authors conclude that the rat is able to synthesize methionine and, probably other metabolites containing the labile methyl group and that vitamin B_{12} and other dietary factors may play a decisive role in the synthesis and utilization of the synthesized labile methyl groups. The possible role of amino acids, particularly glycine and serine, in the synthesis is pointed out.

Laboratory of General Research, University of California
The University of California Cancer Research
Palo Alto, Ca.

- 686 VIVANCO F., JIMENEZ DIAZ, C., and PALA CIO, J. Recent studies on cicerism and nature of CH factor *Rev. Clin. Espan.* 39: 168- Nov 15, 1950 (abstr. J.A.M.A. 146 218, May 12, 1951)

Rats were given a diet in which the only source of protein was the albumin of the chick pea. The rats were divided into five groups, depending upon the dietary supplements given. The first group served as controls, the second received methionine, the third received vitamin B_{12} , the fourth received crude liver extract, and the fifth was given steamed liver extract. All of the animals receiving only the basal diet developed cicerism (the equivalent of lathyrism in humans). Cicerism did not develop in the animals that received methionine or the liver extracts. Cicerism occurred in 87.5 per cent of the animals that received vitamin B_{12} supplements. The experiments show the existence of a vitamin, which the authors call the CH factor in liver extracts. This vitamin is water soluble and thermostable. Apparently the CH factor is necessary in the absence of a certain level of choline or methionine in the diet. The authors believe that the CH factor intervenes in the metabolic processes in such a way as to

correct deficiencies of methionine and choline. The CH factor is considered as being one of the essential components of the animal protein factor. Methionine can be substituted for the CH factor.

687. SAKAMI, W., and WELCH, A. D. *Synthesis of labile methyl groups by the rat in vivo and in vitro* J. Biol. Chem. 187: 379-484 Nov. 1950.

688. BENNETT M. A.: *Utilization of homocysteine for growth in presence of vitamin B₁₂ and folic acid* J. Biol. Chem. 187: 751-756, Dec. 1950.

Rats fed a "labile methyl" free diet containing 0.83 per cent homocysteine as the sole source of sulfur amino acids and the eight B vitamins gradually stopped growing if their intestinal synthesis of folic acid was suppressed by sulfasuxidine. When this leveling had occurred, adding folic acid to the diet caused resumption of growth in some instances, but not in all, and growth eventually stopped even in the animals that had resumed growth. This seemed to indicate that an additional factor was involved. Addition of crystalline vitamin B₁₂ to the vitamin supplement at this point caused a resumption of growth in all animals. After vitamin B₁₂ was discontinued, the animals continued to grow for a short period, after which the curves slowly leveled. Substitution of methionine for homocysteine at the point mentioned above caused a resumption of growth without the addition of B₁₂. The proprietary liver extract used gave growth proportional to its vitamin B₁₂ content, on addition of folic acid. The previous negative results obtained with a proprietary concentrated solution of liver extract were evidently due to folic acid deficiency.

*Lambert Research Institute and
Institute for Cancer Research
Philadelphia, Pa.*

689. LIENER, L. E., and SCHULTZE, M. O. *Liver arginase activity as related to blood urea in acute uremia of new-born rats* J. Biol. Chem. 187: 743-750, Dec. 1950.

Authors summary "The acute uremia observed in new-born rats from mothers maintained on rations containing crude plant materials was accompanied by a significant increase in the activity of liver arginase, compared to rats of normal appearance of the same age in which the maternal diet had been supplemented with fish oil-solubles.

"Liver arginase activity closely paralleled the marked rise in the concentration of urea in the blood which occurred 12 to 24 hours after birth.

"The early postnatal administration of vitamin B₁₂ prevented the abnormal rise in arginase activity and prevented acute uremia in the new-born rat. Under these conditions a higher degree of correlation was found to exist between the arginase activity of the liver and the concentration of urea in the blood."

*University of Minnesota
St. Paul, Minn.*

690. ARNSTEIN H. R. V., and NEUBERGER, A.: *The effect of vitamin B₁₂ on the conversion of glycine to choline* Biochem. J. 48: III, Jan. 1951 (in Proc. Biochem. Soc.)

691. DUBNOFF J.: *Vitamin B₁₂ in methionine formation*, Federation Proc. 10: 178, March 1951.

"It was previously reported (Federation Proc. 9: 166, 1950 [Abstr. 676] and Arch. Biochem. 27: 466, 1950 [Abstr. 680]) that B₁₂ concentrates catalyze the reduction by rat liver slices of homocysteine to homocystine. Further work has shown that crystalline B₁₂ in concentrations as low as 0.0001 γ /ml. is also active. Reduced glutathione markedly increases the reduction of homocystine. Independent evidence confirming this role for B₁₂ in methionine synthesis based on the replaceability of B₁₂ in the B₁₂ or methionine requiring mutant of B. Davis by homocysteine will be reported."

*California Institute of Technology
Pasadena, Calif.*

692. SCHAEFER, A. E., KNOWLES, J. L., and SALMON W. D.: *Influence of vitamin B₁₂ and folic acid on synthesis of choline and methionine by the rat in vivo and in vitro* Federation Proc. 10: 893, March 1951.

"Weanling rats fed a choline-low basal diet containing thoroughly methanol-extracted peanut meal and oxidized casein failed to gain in body weight unless the diet was supplemented with DL-methionine. The effectiveness of various precursors for synthesis of choline and methionine *in vivo* were measured by gain in body weight, protection against renal damage and total carcass choline. Replacing the DL-methionine and choline supplements with DL-homocysteine and various choline precursors, respectively with and without vitamin B₁₂ and/or folic acid indicated that vitamin B₁₂ and folic acid were involved in the synthesis of choline and methionine. When the basal diet was supplemented with homocysteine and ethanolamine, methylation of these compounds by methyl from betaine occurred only when the diet was supplemented with vitamin B₁₂ and folic acid. Glycine and serine or methanol and aminoethanol did not prevent the synthesis of sufficient choline for the support of growth and the prevention of fatal renal hemorrhage in weanling rats. *In vitro* studies were made with liver slices from rats receiving the various deficient diets and from chicks receiving similar diets. The addition of vitamin B₁₂ and folic acid to substrates containing dimethylaminoethanol resulted in a 2 to 4-fold increase in synthesis of free choline."

*Dalhousie Polytechnic Institute
Halifax, N.S.*

693. GOYCO J. A.: *Effect of vitamin B₁₂, DL-methionine and vitamin E on the growth promoting value of Torula yeast protein*, Federation Proc. 10: 191, March 1951.

"Male Wistar rats 28 days old were used. The basal diet contained 18% protein supplied by *Torula* yeast. Trials lasted 4 weeks. Growth promoting value of *Torula* yeast was 1.23. When this ration was supplemented with 200 μ g. B₁₂ per kg. the value was 1.40. The difference was found to be not significant. The incidence of hepatic necrosis in both groups was very high. In a group of animals supplemented with the same amount of B₁₂ and 2 mg. vitamin E per week given separately the growth promoting value was significant, 1.63. No hepatic necrosis was observed in these rats. In another experiment the same basal ration was used except that it contained 0.5% methionine. One group received this ration only a

group the ration separately supplemented with one μg . B_{12} per day and a third group, the ration separately supplemented with one μg . B_{12} per day and 2 mg. vitamin E per week. Growth promoting values were 2.01, 2.54 and 2.77 respectively. The difference between those on methionine alone and those receiving in addition B_{12} was significant. Not a single one developed hepatic necrosis. B_{12} as well as methionine increased food consumption to about the same extent. When fed together the increase in food intake was almost additive. Methionine, B_{12} and vitamin E together step up the growth promoting value of Torula yeast protein to 2.77 a level never observed by us before."

University of Puerto Rico School of Medicine
San Juan, P. R.

- 694 DUNNING, J. S., PAYNE, L. D., and DAY P. L. The influence of folic acid, vitamin B_{12} , and methyl donors on white blood cell production in rats. J. Nutrition 43 525-531, April 1951.

Authors summary "Weanling Sprague-Dawley rats were given a basal methionine-deficient, purified diet. Various groups received this diet with supplements of methionine, choline, or betaine, with and without folic acid and vitamin B_{12} . Rats receiving the basal diet alone developed leucopenia. The leucopenia was prevented by supplements of methionine with or without folic acid and vitamin B_{12} , or by choline or betaine with supplements of folic acid and vitamin B_{12} . Supplements of choline or betaine in the absence of added folic acid and vitamin B_{12} failed to prevent leucopenia."

University of Arkansas School of Medicine
Little Rock, Ark.

- 695 MILLER, E. C., PLESCIA, A. M., MILLER, J. A., and HEIDELBERGER, C. On the metabolism of 3'-methyl-4-dimethylaminoazobenzene-N-methyl- C^{14} in the rat. Cancer Research 11 268-269 April 1951.

"Approximately 70 per cent of the C^{14} from 3'-methyl-4-dimethylaminoazobenzene-N-methyl- C^{14} was expired as carbon dioxide within 48 hours after the administration of a single dose to rats by stomach tube. Approximately 10 per cent of the activity was excreted in the urine and feces, and the remainder was found in the carcass. Of the radioactivity left in the animal after one or several doses, 15-20 per cent was contained in the protein and 8 per cent in the choline, and the specific activity of both of these components was 2-3 times higher in the liver than in the rest of the body. Fifteen to 35 per cent of the activity in the protein was contained in the serine, and all the radioactivity in this amino acid was in the β -position. Since other investigators have shown that formate and formaldehyde are incorporated only into the β -position of serine, at least one N-methyl group from the dye is apparently oxidized through these intermediates or a related one-carbon compound. Most of the radioactivity in the choline was contained in the N-methyl groups, and, although some direct transmethylation cannot be excluded, it seems most likely that the activity was derived from formate or formaldehyde. Rats deficient in either folic acid or vitamin B_{12} incorporated much less radioactivity into the liver serine and choline than control rats supplemented with both of these vitamins."

University of Minnesota
Minneapolis, Minn.

- 696 SCHAEFER, A. E., and KNOWLES, J. L. Influence of vitamin B_{12} and folic acid on the synthesis of choline and methionine by the rat. Proc. Soc. Exper. Biol. & Med. 77 655-659 Aug. 1951.

Authors summary "The role of vit. B_{12} and folic acid in the biosynthesis of choline and methionine has been studied and the following conclusions are warranted: 1. In the presence of adequate methyl (from choline) vit. B_{12} alone was as effective for methionine synthesis as when combined with folic acid. When fed alone, folic acid was ineffective. 2. In the presence of a limited supply of methyl groups, both folic acid and vit. B_{12} were essential for the normal development of weanling rats. 3. Vit. B_{12} and folic acid were essential for the synthesis of choline and of methionine from aminomethanol, homocysteine, and a limited supply of betaine. 4. As a methyl donor for the synthesis of methionine from homocysteine, betaine was at least as effective as choline, mole per mole. 5. Under the experimental conditions employed, the rate of synthesis of the methyl group or utilization of methanol for the methylation of homocysteine to methionine and aminomethanol to choline was not fast enough to support normal development of weanling rats."

Alabama Polytechnic Institute
Tuscaloosa, Ala.

- 697 HALE, O. M., and SCHAEFER, A. E. Choline requirement of rats as influenced by age, strain, vitamin B_{12} and folic acid. Proc. Soc. Exper. Biol. & Med. 77 633-636 Aug. 1951.

Authors summary "(1) Weanling rats of the Sprague-Dawley (SD) strain have a lower choline requirement for protection against renal hemorrhage and a higher requirement for maintenance of normal liver fat than was observed in rats of the Alabama Experiment Station (AES) strain. (2) Forty-two-day-old rats of either strain grew rapidly and failed to develop hemorrhagic kidneys when they were fed the choline-deficient basal diet supplemented with only vit. B_{12} and folic acid. However liver fat of the SD strain rats was 35% (dry basis) as compared to 62% for the AES strain rats. (3) The addition of vit. B_{12} and folic acid to the basal diet supplemented with suboptimal choline chloride markedly suppressed the accumulation of fat in the liver, reduced the incidence of renal damage and increased the choline content of the livers of the SD strain of rats. Liver fat values averaging 12.8% (dry basis) were obtained with a 0.20% choline chloride supplement in the presence of vit. B_{12} and folic acid as compared to a requirement of 0.40% choline chloride in the absence of vit. B_{12} and folic acid."

Alabama Polytechnic Institute
Tuscaloosa, Ala.

- 698 WILLIAMS, J. N.: Further studies on choline oxidase factors. J. Biol. Chem. 192 81-84, Sept. 1951.

Folic acid, L-leucovorin citrovorum factor (LCF) vitamin B_{12} , and ascorbic acid singly and in combination markedly stimulate choline oxidation in liver homogenates of rats fed aminopterin.

The results indicate that folic acid or LCF vitamin B_{12} and ascorbic acid are important in maintaining choline oxidase.

- 699 SAHASRABUDHE, M. R., and LAKSHMINARA YAN RAO M. V. *Effect of vitamin B₁₂ on the synthesis of protein and nucleic acids in the liver* Nature 168 605-606, Oct. 6, 1951

Vitamin B₁₂ stimulates both protein and nucleic acid synthesis in the liver

"The greater regenerations of liver protein in animals receiving B₁₂ is presumably due to its effect in enhancing amino acid utilization.

"We believe these results are the first experimental proof of the direct intervention of vitamin B₁₂ in mammalian nucleic acid metabolism."

700. BENNETT M. A., JORALEMON J., and HALPERN P. E. *The effect of vitamin B₁₂ on rat growth and fat infiltration of the liver* J. Biol. Chem. 193 285-291 Nov 1951

Crystalline vitamin B₁₂ or equivalent amounts of this vitamin (as liver or APF concentrate) will enable rats to grow at practically identical rates on a "labile methyl"-free homocysteine diet containing folic acid and to maintain normal livers.

When homocysteine is replaced by methionine in the diet, the rats grow at essentially identical rates either in the absence or presence of crystalline B₁₂ or equivalent amounts of other sources of the vitamin.

701. LIENER, I. E., and SCHULTZE, M. O. *The excretion of N⁵-methylisocotinine and creatinine as influenced by vitamin B₁₂ and various methyl donors*, J. Nutrition 46 223-237 Feb. 11, 1952.

Authors summary "Young vitamin B₁₂-deficient rats were fed a labile-methyl free ration with the following supplements in the presence or absence of vitamin B₁₂: none methionine choline homocysteine homocysteine plus choline, betaine, or formate. The urinary excretion of N⁵-methylisocotinine and creatinine was determined following an intraperitoneal dose of nicotinamide or guanidoacetic acid.

"Only when the basal ration was supplemented with homocysteine in the presence of choline, betaine, or vitamin B₁₂ were the growth and the excretion of N⁵-methylisocotinine similar to those observed with the methionine-supplemented ration. The administration of vitamin B₁₂ effected a further improvement in growth and increase in N⁵-methylisocotinine excretion when the diet contained homocysteine plus choline or betaine. Formate in the absence of vitamin B₁₂ was ineffective as a methyl donor in homocysteine.

"When the basal ration contained homocysteine, the administration of vitamin B₁₂ or additional supplements with choline caused an increase in growth and excretion of creatinine comparable to the increase obtained with the methionine-supplemented ration. Choline alone, although incapable of supporting growth, effected a similar increase in creatinine output.

"The implication of these results with respect to the role of the vitamin B₁₂ in the biosynthesis of methionine has been discussed."

University of Minnesota
St. Paul, Minn.

702. LANGE MANN H., and KENSLE, C. J. *Methionine formation in liver homogenates* Federation Proc. 11 366, March 1952.

"Conditions have been established for the measurement of methionine formation from homocysteine and choline, betaine or dimethylthetin in liver homogenates which yield higher Q methionine values than those previously reported by others. Evidence obtained using succinate to saturate proton and electron acceptors present in tissue homogenates supports Dubnoff's suggestion that in the rat choline must be oxidized before its methyl group is available for methionine synthesis. The use of succinate in the system increased the values obtained when betaine was the methyl donor but greatly reduced the values when choline was the methyl donor. The dimethylthetin-homocysteine transmethylation activity of mouse liver was twice that of rat liver. Rabbit, guinea pig and chick liver was less active than rat liver. Chick liver activity being less than 1/2 that of rat liver. Betaine transmethylation activity did not parallel dimethylthetin transmethylation activity among the different species. Rat and mouse liver tumors showed little or no transmethylation activity. In chicks deficient in folic acid or Vitamin B₁₂, transmethylation activity was not reduced. The folic acid deficient chicks yielded appreciably higher values than their supplemented controls."

Cornell University Medical College
New York, N. Y.

- 703 JOHNSON C. A., KIRCH, E. R., and BERGEIM, O. *Effects of methionine, ethionine and of certain antibiotics on liver iron*, Federation Proc. 11 236, March 1952.

"Hemochromatosis has been noted in individuals on deficient diets and Kinney Hepstead and Finch (J. Exper. Med. 90 137 1949) observed that methionine added to diets of corn grain led to an increase of liver iron in rats. We have studied the effect on young, growing rats and adult rats on adequate casein containing diets of the addition of 8% methionine over periods of 3-7 weeks. In the younger rats on moderate iron intakes methionine increased liver iron significantly. Spleen iron was also increased. Phenylalanine did not have this effect. Ethionine brought about a decrease in liver iron. Vitamin B₁₂ led to an increase in spleen but not in liver iron. High doses of neomycin and streptomycin increased liver iron. In adult animals on high iron diets (6% ferric citrate) methionine increased liver and spleen iron. Ethionine alone produced a slight rise in liver iron and a decline in the spleen. Given with methionine it counteracted the effects of methionine on liver and spleen iron. With increases in total iron in the livers the amounts present in HCl extracts as ferrous iron increased. However the ratios of ferrous iron to total iron markedly decreased. Ratios of ferrous to ferrous iron in cecal contents showed no clear relation to liver values. Some alterations were noted in acid extractable sulfhydryl compounds of the liver."

Dunsmuir of Illinois
Chicago, Ill.

- 704 ROSE, I. A., and SCHWEIGERT B. S. *Effect of vitamin B₁₂ on nucleic acid metabolism of the rat*, Proc. Soc. Exper. Biol. & Med. 79 541-544, March 1952.

This study demonstrates the effect of a vitamin B₁₂ deficiency on the amounts of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) on rat organs, and on the rate of incorporation of N₁₅ labeled glycine into the purines of RNA. The authors' summary reads as follows:

1. The amounts of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in the livers of rats fed a vit. B₁₂ deficient diet were significantly lower per g. of fresh or of dry liver or of liver nitrogen than those for the supplemented rats. No difference was observed, however, in the amounts of RNA and DNA per cell for the deficient or supplemented rats. The amount of nucleic acids per g. of spleen or kidney was the same for the deficient and supplemented group. 2. Other studies indicated that the rates of incorporation of N₁₅ glycine into the purines of RNA were lower for the deficient animals. 3. These studies suggest that in a vit. B₁₂ deficiency the reduced rate of nucleic acid synthesis in the liver reduced the rate of cell divisions."

University of Chicago
Chicago, Ill.

- 705 STEKOL, J., WEISS S., and WEISS, K.: *Vitamin B₁₂ and folic acid in the synthesis of choline in the rat*. Arch. Biochem. & Biophysics 36 5-10, 1952 (abstr. Blood 7: 857 Aug. 1952)

"The results are interpreted as indicating the involvement of vitamin B₁₂ or of its physiologic derivative in the synthesis of serine from glycine. Folic acid or its physiologic derivative is involved in the synthesis of both moieties of choline from serine, and, through serine, from glycine."

Lankenau Hospital Research Institute, and
University for Cancer Research
Philadelphia, Pa.

706. MORGAN A. F., and LEWIS E. M. *Modification of choline deficiency by simultaneous pantothenic acid deficiency* Federation Proc. 11 451 March 1952.

"The effect of choline deficiency upon rats fed normal and pantothenic acid-deficient diets was observed from the 14th to the 56th day of life. In the pantothenic acid-deficient and doubly deficient animals the liver fat remained normal or low and adrenal cholesterol much lower than in the normal and choline-deficient groups even when pair-fed. When vitamin B₁₂ was given the liver fats were normal in all cases but adrenal cholesterol remained markedly depressed in the pantothenic acid-deficient rats. Neither the addition of 1% cholesterol to the diet nor the daily injection of adrenocortical extract increased the adrenal cholesterol or the liver fat of animals deprived of pantothenic acid. Blood and serum lipid phosphorus levels were slightly raised in the latter groups. Daily injection of 2 mg. ACTH for 5 days before autopsy produced increases in liver fat of normal animals but none in pantothenic acid-deficient groups. The adrenal cholesterol of ACTH treated animals was somewhat increased but in all the pantothenic acid-deficient rats these levels were less than half that of the normal animals. The thymus weights were greatly reduced in all the pantothenic acid-deficient groups whether ACTH treated or not. Thus, lipid metabolism of pantothenic acid-deficient rats was severely deranged as shown by failure of fat

deposition in the liver, loss of adrenal cholesterol, and rise in serum lipid phosphorus. An increase in lipid utilization may occur in this deficiency state."

University of California
Berkeley, Calif.

- 707 SCHEPARTZ, B., and NADEL, E. M.: *Effect of ascorbic acid, folic acid and vitamin B₁₂ upon tyrosine oxidation in acetone powder preparations of guinea pig liver* Federation Proc. 11 425, March 1952.

"Acetone powder preparations of guinea pig livers when supplemented with a ketoglutarate were found capable of converting L-tyrosine to acetoneacetate. As previously reported for powders prepared from rat livers, the catabolism of tyrosine required an initial transamination, with a ketoglutarate as the acceptor of the amino group (B. Schepartz, J. Biol. Chem. 193: 293, 1951). Enzymatic activity was reduced 50% in livers of ascorbic animals. This deficiency was remedied by the *in vitro* addition of ascorbic acid. Decreased food consumption by ascorbic animals was not the cause of the decline in enzymatic activity for acetone powders prepared from livers of fasted animals that had received oral supplements of ascorbic acid had normal levels of activity. *In vitro* additions of folic acid or of vitamin B₁₂ did not restore the activity of preparations from ascorbic animals. Aging of the acetone powders prepared from normal livers caused a decrease in the activity of these powders to levels obtained in preparations from ascorbic animals. Normal activity was restored [by] *in vitro* supplementation with ascorbic acid. The data confirm the reports of other laboratories on the importance of ascorbic acid in tyrosine metabolism."

Jefferson Medical College
Philadelphia, Pa.
National Cancer Institute
Bethesda, Md.

708. EDWARDS, C. H., and CARTER, R. T. *Interrelationships between vitamin B₁₂ and methionine in metabolism*, Federation Proc. 12 413, March 1953.

"Research conducted during the past year suggests that vitamin B₁₂ and methionine are intimately related in the metabolism of rats partially depleted of protein reserves. After ad-libitum feeding for 6 days of a diet low in nitrogen but adequate in other nutrients, adult male rats were force-fed a basal ration containing eight essential amino acids (methionine and arginine-free) for a 9-day interval (period I). The animals were divided into groups of 6 and fed daily for 9 days (period II) the basal amino acid diet with either a) no supplement, b) 25 mg. methionine, c) 0.4 micrograms vitamin B₁₂, d) 25 mg. methionine and 0.4 micrograms vitamin B₁₂, or e) 50 mg. methionine. Nitrogen balances were determined during the last 5 days of periods I and II. Animals receiving a supplement of 25 mg. methionine were in negative nitrogen balance and had high concentrations of liver fat. When the diet was supplemented with vitamin B₁₂ and 25 mg. methionine, the animals were in nitrogen equilibrium and the concentrations of liver fat were normal. In the absence of methionine, animals receiving vitamin B₁₂ were in negative nitrogen balance. When 50 mg. methionine were fed, nitrogen balance was achieved, but concentrations of liver fat were abnormally high.

Methionine alone, in concentrations which maintained nitrogen balance, stimulated a 50% increase in the production of hemoglobin."

*Yadav, Lachman
Yadav, A.*

Swine

- 709 CUNHA, T. J., HOPPER, H. H., BURNSIDE, J. E., PEARSON, A. M., GLASSCOCK, R. S., and SHEALY, A. L.: Effect of vitamin B₁₂ and AFP supplement on methionine needs of the pig. *Arch. Biochem.* 510-512, Oct. 1919 (Letter to Editor)

EXCRETION ABSORPTION RETENTION

710. CHOW B. F., BARROWS, L., and LANG C. Microbiological activity of vitamin B₁₂ in urine of normal rats following oral and subcutaneous administration of this vitamin. *J Nutrition* 42 405-414, Nov 1950.

- 711 CHESTERMAN D. C., CUTHBERTSON W. F. J., and PEGLER, H. F. Vitamin B₁₂ excretion studies. *Biochem. J* 48 B-31, April 1951 (in *Proc. Biochem. Soc.*)

712. BARBEE, K. W., and JOHNSON H. C. Metabolism of radioactive vitamin B₁₂ by the rat. *Proc. Soc. Exper Biol. & Med.* 76 720-721 April 1951

Radioactive vitamin B₁₂ has been given orally or subcutaneously to 4 adult white rats. The radioactivity (entirely B₁₂) was excreted primarily in the urine following injection, while the radioactivity (B₁₂ plus inorganic cobalt) was excreted primarily in the feces following oral administration.

713. ABBOTT L. D., Jr. Biochemical changes induced by vitamin B₁₂. *Federation Proc.* 10 153, March 1951.

"Nitrogen and phosphorus balances before and after injection of vitamin B₁₂ in rabbits on both unrestricted and constant dietary intake have been studied. In rabbits on unlimited intake of Rabbit Chow Checkers, injection of B₁₂ (5 µg./day for 5 or 6 days) was accompanied by marked positive nitrogen balance. The animals ate more and retained more nitrogen than in preceding control periods. Phosphorus retention also occurred. The ratio of P:N retained under the influence of B₁₂ approximated Benedict's P:N ratio for protoplasm (1:14). When B₁₂ administration was stopped balances gradually decreased to former levels. In animals on a constant intake containing 2.5 gm. N per day slight positive nitrogen balance occurred under the influence of B₁₂ even though additional food was not available during this period. On a constant diet of oats and cabbage which furnished a low N intake, urinary excretion of nitrogen, urea, amino acids and phosphorus diminished during B₁₂ administration. These results indicate that in the normal rabbit under these conditions increased utilization of nitrogen and phosphorus occurred."

*Medical College of Physics
Baltimore, Pa.*

- 714 BENARD H., GAJDOS A., and GAJDOS-TÖRÖK, M.: Continuous metabolism w/ haemoglobin in the red cells of peripheral blood, *Nature* 167 989-990, June 16, 1951

Subcutaneous injection of a single dose of 15 mg. of folic acid or of 20 mcg of vitamin B₁₂ induces in rabbits, in 6 to 24 hours, a notable erythrocytosis. But at the same time the hemoglobin value, instead of increasing, remains at its initial level, with a consequent marked lowering of the color index (0.80-0.93 to 0.53-0.75)

A concept of continuous metabolism of hemoglobin is outlined. The authors have observed the content of hemoglobin of the red cell to be variable during its life protoporphyrin derived from degradation of hemoglobin is not utilized for the resynthesis of hemoglobin instead new protoporphyrin has to be synthesized.

Paris, France

- 715 DE MELLO R. P. Effect of vitamin B₁₂ folic acid and nicotinamide on urinary coproporphyrin excretion in photosensitized rabbits. *Proc. Soc. Exper Biol. & Med.* 77 744-747 Aug. 1951.

The author has previously shown that the photodynamic effect of Rose Bengal and ultraviolet rays increases urinary coproporphyrin within 24 to 48 hours in rabbits. In the present paper the effect of vitamin B₁₂, folic acid, and nicotinamide on this increase was studied. Folic acid (5, 15, or 45 mg.) and vitamin B₁₂ (5, 10, 15 or 30 mcg.) were injected 10 minutes before the administration of Rose Bengal and nicotinamide was given in three doses totaling 200 to 300 mg. one dose was given before and two doses after administration of Rose Bengal. Folic acid in the lower doses partially and in the highest dose completely inhibited increase of urinary coproporphyrin after administration of Rose Bengal and exposure to ultraviolet rays. Vitamin B₁₂ almost completely prevented the increase of urinary coproporphyrin. The combination of folic acid and B₁₂ was no more effective than the substances individually. Nicotinamide decreased the coproporphyrinuria caused by ultraviolet rays, but did not prevent it. Since these three substances are all intimately related to cellular metabolism, it seems highly likely that the increases of urinary coproporphyrin under the experimental conditions described are likewise so related, but whether to the metabolism of the bone marrow or to some other system of cells is not clear

*University of Minnesota Hospital
St. Paul, Minn.*

- 716 KULWICH, R., STRUCLIA, L., and PEARSON P. B.: The effect of coprophagy on the excretion of B vitamins by the rabbit. *J Nutrition* 49 639-645 April 10, 1953.

Authors summary "Rabbits that are collared to prevent coprophagy or consumption of the soft feces excrete approximately 29% more feces than are recovered when coprophagy is not prevented. The difference is essentially accounted for by the soft feces excreted. The soft feces contain between three and 4 times more niacin and riboflavin per gram than do the hard feces. Pantothenic acid is about 6 times greater in the soft feces and vitamin B₁₂ between two and three times the level

the hard feces. On the basis of the difference in the fecal excretion of the vitamins or their recovery in the feces of the rabbits while collared as compared with the amounts recovered while the animals were not collared, it can be calculated that coprophagy provides the rabbit with about 83% more niacin, 100% more riboflavin, 165% more pantothenic acid and 42% more vitamin B₁₂ than would be available if the soft feces were not consumed.

"The amount of vitamin B₁₂ recovered in the feces of rabbits fed a diet low in this vitamin was 221 fold greater than the intake. Values are given for the vitamin B₁₂ content of the liver, kidney and spleen of the rabbit."

U. S. Department of Agriculture
Washington, D. C.

- 717 NUTRITION REVIEWS: *The utilization of vitamin B₁₂*. Nutrition Reviews 10 219- July 1952 (abstr J Am. Dietet. A. 28 1078, Nov 1952)

"Data have seemed to indicate that vitamin B₁₂ is apparently not absorbed in any large quantity in normal individuals. From recent evidence, however, it appears that in dogs, absorption of the vitamin is substantially proved on the basis of blood levels. When orally administered to rats, the vitamin showed evidence of absorption and its level in the diet had a marked effect upon its quantity in the animals' milk. In studying the influence of the diet as a whole upon the rat's absorption of vitamin B₁₂, it appeared that pork may contain a factor or factors that somehow interfere with normal utilization of the vitamin. Cobalt injections produced an expected increase in red blood cells but failed to influence the storage of vitamin B₁₂ in the liver. The polycythemia inducing action of cobalt cannot be explained on the basis of an increased formation of the vitamin in the rat. Present evidence indicates that absorption varies considerably and is affected by the general diet."

718. ROSENTHAL, H. L., and HAMPTON J. K. *Absorption of vitamin B₁₂ in dogs*, Federation Proc. 12 423, March 1953.

"A procedure to determine vitamin B₁₂ activity in blood serum with *Lactobacillus leichmanii* (ROSENTHAL AND SARETT J. B. C. 190 433, 1952) was applied to the study of absorption of vitamin B₁₂ from the intestinal tract of dogs. Crystalline vitamin B₁₂ was placed in the ligated and unligated duodenum of anesthetized dogs and samples of portal and peripheral blood were obtained over an eight hour period. In other dogs, vitamin B₁₂ was placed in the stomach ligated at the pyloric sphincter. Serum vitamin B₁₂ activity of eleven fasted dogs was 0.25 \pm 0.10 μ g./ml. which is similar to that of normal human subjects, 0.20 \pm 0.03 μ g./ml. In two dogs with ligated duodenum given 50 μ g. vitamin B₁₂/kg., serum activity increased from fasting values to maxima of 3.4 μ g./ml. and 6.5 μ g./ml. after 4 and 8 hours respectively. With 10 μ g./kg., serum activity increased slightly in one dog and rose to 0.95 μ g./ml. in another. In one dog with

an unligated duodenum, maximum serum activity after 50 μ g./kg. was 1.68 μ g./ml. In two dogs with stomachs ligated, no rise in serum activity followed 50 μ g./kg. In dogs subjected to operation but given no vitamin B₁₂, serum activity remained constant. Findings in portal blood followed the same pattern as in peripheral blood. These data show that vitamin B₁₂ is absorbed primarily in the duodenum and not in the stomach."

Tulane University School of Medicine
New Orleans, La.

- 719 LANG, C. A., and CHOW B. F. *Retention of vitamin B₁₂ after administration to subjects of different ages*, Federation Proc. 11 88, March 1952.

"A small portion of the vitamin B₁₂ administered parenterally to normal adult rats in dosages of 1.2 μ g. appears in the urine, and the remainder is retained by certain tissues and concentrated in organs such as liver, kidneys, and pancreas. Experiments were carried out to compare the urinary excretion and retention of this vitamin after injection into rats of different ages. For this purpose, 15 2-month-old and 16 14-month-old male rats raised on our stock diet fortified with 2% decaerated whole liver were placed in individual metabolism cages. They were divided into 4 sub-groups and given a single subcutaneous injection of crystalline vitamin B₁₂ tagged with Co⁶⁰ in dosages of 1.8 μ g./rat. Urine specimens (0-24 and 24-28 hours after administration and in some instances pre-injection samples) were collected and assayed for vitamin B₁₂ both by a microbiological method and by radioactivity measurement. Fourteen days after injection the animals were sacrificed, and the above-mentioned organs were removed for the determination of radioactivity after conversion into inorganic Co⁶⁰ salts. Our results demonstrate: 1) At a given dosage the excretion of vitamin B₁₂ was essentially the same for both age groups if expressed as μ g./animal but was significantly different if expressed as μ g./100 gm. body weight. This is due to the fact that the average weight of the older animals (457 gm.) was approximately twice that of the young ones (248 gm.) 2) Retention was related both to dosage and to age. Similar excretion studies were made using human male subjects of 2 different age groups, namely 25-40 years of age and 65 or greater who were of essentially the same body weight. Our preliminary results again indicated that the young individuals excreted more."

Iowa Hopkins University
Baltimore, Md.

720. SIEBERT G., LANG K., and LANG H. *Investigations on the metabolism in cell nuclei. Vitamin B₁₂ and cobalt in cell nuclei*, Biochem. Z. 321 543-548, May 1951.

The fact that the vitamin B₁₂ and cobalt content of cell nuclei is very low and that following intravenous injection of Co⁶⁰ the nuclei absorb only half as much cobalt as tissues, indicates that B₁₂ and cobalt have no significant part in the metabolism of cell nuclei.

VITAMIN B₁₂ CONTENT ACTIVITY

- 721 COUCH, J. R., OLCESE, O., WITTEN, P. W., and COLBY, R. W.: *Vitamin B₁₂ content of blood from various species*, *Am. J. Physiol.* 163: 77-80, Oct. 1950.

The authors determined the vitamin B₁₂ content of whole blood from a number of species as measured by Lactobacillus leichmannii 4749. Vitamin B₁₂ activity for some of the species was determined also after autoclaving of the whole blood samples at pH 12. Values obtained are listed in the accompanying table.

SOURCE OF BLOOD SAMPLES	B ₁₂ CONTENT (mcg./cc. blood)		B ₁₂ ACTIVITY (mcg./cc. blood) destroyed by autoclaving
	Before autoclaving	After autoclaving	
human	0.8	—	—
dog	0.9	—	—
cotton rat	3.6	0.0	3.6
white rat	0.8	0.0	0.7
American albino mice	2.3	0.0	2.3
Swiss albino mice	1.2	0.0	1.2
calves (dry lot fed)	0.9	0.0	0.9
cow (pasture fed)	0.5	—	—
sheep	0.7	0.0	0.7
goat	0.7	0.0	0.7
pig	1.0	—	—
horse	2.1	—	—
rabbit	10.1	0.0	10.1
turtle	6.6	4.5	2.0
chick	5.6	4.3	1.0
turkey	5.3	3.8	1.5

It can be observed from the table that the vitamin B₁₂ activity of blood in most of the species was destroyed by autoclaving at pH 12.0. In the turtle, chicken and turkey which had a vitamin B₁₂ activity appreciably higher than that of the other species tested except for the rabbit, only a portion of the B₁₂ activity was destroyed. This is believed to be due to the fact that these animals have nucleated erythrocytes; therefore a part of the L. leichmannii activity is due to thymidine. The exceptionally high B₁₂ content of rabbit blood is believed attributable to the habit of nocturnal coprophagy.

From *Physiological Experiments Section*
College Station, Texas

- 722 PFANDER, W. H., DIETRICH, L. S., MONSON, W. J., HARPER, A. E., and ELVEHJEM, C. A.: *Citrovorum factor, vitamin B₁₂, and folic acid activity of whole blood of several species*, *Proc. Soc. Exper. Biol. & Med.* 79: 219-221, Feb. 1952.

The citrovorum factor (LCF), vitamin B₁₂, and folic acid (PCA) content of whole blood of several species (calf, chicken, sheep, ox, and rat in various stages of B₁₂ depletion) was studied. Little or no free LCF active material was demonstrated, but demonstrable amounts of free Streptococcus faecalis activity were found. Release of LCF and PCA during autolysis was dependent on the pH of the reaction mixture: optimum values were obtained at pH 4.5 or 6.5 to 7.0, depending on the species studied. LCF was low in hyperthyroid vitamin B₁₂-deficient rats but levels similar to those in normal rats after fasting

were found in similar rats given 3 mcg. of vitamin B₁₂ a day; PCA activity was not appreciably changed by administration of vitamin B₁₂ but the B₁₂ level was significantly increased.

University of Wisconsin
Madison, Wis.

- 723 DOCTOR, V. M., and COUCH, J. R.: *Bioautographic analysis of blood for growth factors active for L. leichmannii* 4797 *Proc. Soc. Exper. Biol. & Med.* 81: 222-224, Oct. 1952.

Aqueous extracts of autolyzed blood samples from various species were chromatographed on paper strips and analyzed by bioautographic procedures.

Chicks

- 724 COUCH, J. R., OLCESE, O., and GERMAN, H. L.: *Vitamin B₁₂ content of chick tissues as influenced by diet*, *Federation Proc.* 9: 855, March 1950.

- 725 COUCH, J. R., and OLCESE, O.: *The vitamin B₁₂ content of chick tissues as influenced by the diet*, *J. Nutrition* 42: 337-346, 1950.

- 726 DARBY, H. H.: *Metabolism of cobalt 56 in the hen's ovary*, *Federation Proc.* 9: 856, March 1950.

"With the publication of the fact that cobalt is incorporated in vitamin B₁₂ and that B₁₂ is found in the droppings of chickens, it became of interest to study the fate of cobalt fed to hens. It had already been shown that increased growth could be obtained by injecting small amounts of B₁₂ into developing eggs. This would lead one to believe that there was a shortage of B₁₂ in the average hen's diet. Cobalt 56 was fed in pellet form to 2 hens and the eggs collected for some 3 weeks thereafter. The eggs were tested as a whole first for radioactivity and then they were separated into shell, albumen, and yolk. The amount of cobalt in each fraction was ascertained. The shell and the albumen followed a typical excretory pattern. There was a rise of radioactive cobalt in the shell and the albumen on the 4th-5th day followed by a fall to almost zero by the 10th day. The rise of radioactive cobalt in the yolk, however, was somewhat slower and although there was a high level for some 10 days, no fall of cobalt was found similar to that in the albumen and the shell. It leads one to believe that the yolk of the egg follows a different pattern of metabolism than does that of the shell and the albumen. From these experiments it seems that cobalt once laid down in the yolk is retained in these formative eggs."

Corvallis Extension of Washington
Washington, D. C.

- 727 JACKSON, J. T., MACHLIN, L. J., BRANDENBURGER, E. A., KELLOGG, W. L., and DENTON, C. A.: *Retention of Co 60 labeled vitamin B₁₂ in chickens*, *Proc. Soc. Exper. Biol. & Med.* 83: 221-222, June 1953.

Authors' summary: "The results indicate that over 50% of the injected Co 60 activity is retained for as long as 12 weeks in chicks hatched from eggs injected with the

the hard feces. On the basis of the difference in the fecal excretion of the vitamins or their recovery in the feces of the rabbits while collared as compared with the amounts recovered while the animals were not collared, it can be calculated that coprophagy provides the rabbit with about 83% more niacin, 100% more riboflavin, 165% more pantothenic acid and 42% more vitamin B₁₂ than would be available if the soft feces were not consumed.

"The amount of vitamin B₁₂ recovered in the feces of rabbits fed a diet low in this vitamin was 221 fold greater than the intake. Values are given for the vitamin B₁₂ content of the liver, kidney and spleen of the rabbit."

U. S. Department of Agriculture
Washington, D. C.

- 717 NUTRITION REVIEWS: The utilization of vitamin B₁₂. Nutrition Reviews 10 219- July 1952 (abstr. J. Am. Dietet. A. 28 1078, Nov 1952)

"Data have seemed to indicate that vitamin B₁₂ is apparently not absorbed in any large quantity in normal individuals. From recent evidence, however, it appears that in dogs, absorption of the vitamin is substantially proved on the basis of blood levels. When orally administered to rats, the vitamin showed evidence of absorption and its level in the diet had a marked effect upon its quantity in the animals' milk. In studying the influence of the diet as a whole upon the rat's absorption of vitamin B₁₂, it appeared that pork may contain a factor or factors that somehow interferes with normal utilization of the vitamin. Cobalt injections produced an expected increase in red blood cells but failed to influence the storage of vitamin B₁₂ in the liver. The polycythemia-inducing action of cobalt cannot be explained on the basis of an increased formation of the vitamin in the rat. Present evidence indicates that absorption varies considerably and is affected by the general diet."

718. ROSENTHAL, H. L., and HAMPTON J. K.: Absorption of vitamin B₁₂ in dogs. Federation Proc. 12 428, March 1953

"A procedure to determine vitamin B₁₂ activity in blood serum with *Lactobacillus leichmannii* (ROSENTHAL AND SARETT / B. C. 199: 433, 1952) was applied to the study of absorption of vitamin B₁₂ from the intestinal tract of dogs. Crystalline vitamin B₁₂ was placed in the ligated and unligated duodenum of anaesthetized dogs and samples of portal and peripheral blood were obtained over an eight hour period. In other dogs, vitamin B₁₂ was placed in the stomach ligated at the pyloric sphincter. Serum vitamin B₁₂ activity of eleven fasted dogs was 0.25 ± 0.10 $\mu\text{g./ml.}$ which is similar to that of normal human subjects, 0.20 ± 0.08 $\mu\text{g./ml.}$ In two dogs with ligated duodenum given 50 $\mu\text{g.}$ vitamin B₁₂/kg., serum activity increased from fasting values to maxima of 8.4 $\mu\text{g./ml.}$ and 6.5 $\mu\text{g./ml.}$ after 4 and 8 hours respectively. With 10 $\mu\text{g./kg.}$, serum activity increased slightly in one dog and rose to 0.95 $\mu\text{g./ml.}$ in another. In one dog with

an unligated duodenum, maximum serum activity 50 $\mu\text{g./kg.}$ was 1.68 $\mu\text{g./ml.}$ In two dogs with a ligated, no rise in serum activity followed 50 $\mu\text{g.}$ dogs subjected to operation but given no vitamin serum activity remained constant. Findings in blood followed the same pattern as in periphery. These data show that vitamin B₁₂ is absorbed in the duodenum and not in the stomach."

Tulane University School of Medicine
New Orleans, La.

- 719 LANG, C. A., and CHOW B. F. Retention of vitamin B₁₂ after administration to subjects of different ages. Federation Proc. 11 88, March 1952

"A small portion of the vitamin B₁₂ administered parenterally to normal adult rats in dosages of appears in the urine, and the remainder is retained in certain tissues and concentrated in organs such as kidneys, and pancreas. Experiments were carried out to compare the urinary excretion and retention of the vitamin after injection into rats of different ages. For purpose, 15 2-month-old and 16 14-month-old rats raised on our stock diet fortified with 2% de whole liver were placed in individual metabolism cages. They were divided into 4 sub-groups and given a subcutaneous injection of crystalline vitamin B₁₂ with Co⁶⁰ in dosages of 1.8 $\mu\text{g./rat.}$ Urine samples (0-24 and 24-28 hours after administration and instances pre-injection samples) were collected and assayed for vitamin B₁₂ both by a microbiological and by radioactivity measurement. Fourteen days after injection the animals were sacrificed, and the abdominal organs were removed for the determination of radioactivity after conversion into inorganic Co⁶⁰. Our results demonstrate 1) At a given dosage the retention of vitamin B₁₂ was essentially the same for all groups if expressed as $\mu\text{g./animal}$ but was significantly different if expressed as $\mu\text{g./100 gm. body weight}$ is due to the fact that the average weight of the animals (457 gm.) was approximately twice that of young ones (248 gm.). 2) Retention was related to dosage and to age. Similar excretion studies were made using human male subjects of 2 different ages (namely 25-40 years of age and 65 or greater years of age) of essentially the same body weight. Our preliminary results again indicated that the young individuals excrete more."

Tulane University School of Medicine
New Orleans, La.

720. SIEBERT G., LANG K., and LANG H. Investigations on the metabolism in cell nuclei. P. B₁₂ and cobalt in cell nuclei. Biochem. Z. 321 548, May 1951.

The fact that the vitamin B₁₂ and cobalt in cell nuclei is very low and that following intracellular injection of Co⁶⁰ the nuclei absorb only half as much cobalt as tissues, indicates that B₁₂ and cobalt have a significant part in the metabolism of cell nuclei.

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- 721 COUCH, J. R., OLCESE, O., WITTEN P. W., and COLBY R. W. *Vitamin B₁₂ content of blood from various species*, Am. J. Physiol. 163 77-80 Oct. 1950.

The authors determined the vitamin B₁₂ content of whole blood from a number of species as measured by *Lactobacillus leichmannii* 4749. Vitamin B₁₂ activity for some of the species was determined also after autoclaving of the whole blood samples at pH 12. Values obtained are listed in the accompanying table:

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sheep	0.7	0.0	0.7
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pig	1.0	—	—
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It can be observed from the table that the vitamin B₁₂ activity of blood in most of the species was destroyed by autoclaving at pH 12.0. In the turtle, chicken and turkey which had a vitamin B₁₂ activity appreciably higher than that of the other species tested except for the rabbit, only a portion of the B₁₂ activity was destroyed. This is believed to be due to the fact that these animals have nucleated erythrocytes; therefore a part of the *L. leichmannii* activity is due to thymidine. The exceptionally high B₁₂ content of rabbit blood is believed attributable to the habit of nocturnal coprophagy.

From *Lactobacillus leichmannii* Strain
College Station, Texas

- 722 PFANDER, W. H., DIETRICH, L. S., MONSON W. J., HARPER, A. E., and ELVEHJEM, C. A. *Citrovorum factor vitamin B₁₂ and folic acid activity of whole blood of several species*, Proc. Soc. Exper. Biol. & Med. 79 219-221 Feb. 1952.

The citrovorum factor (LCF) vitamin B₁₂ and folic acid (PCA) content of whole blood of several species (calif, chicken, sheep, ox, and rat in various stages of B₁₂ depletion) was studied. Little or no free LCF active material was demonstrated, but demonstrable amounts of free *Streptococcus faecalis* activity were found. Release of LCF and PCA during autolysis was dependent on the pH of the reaction mixture optimum values were obtained at pH 4.5 or 6.5 to 7.0, depending on the species studied. LCF was low in hyperthyroid vitamin B₁₂-deficient rats but levels similar to those in normal rats after fasting

were found in similar rats given 3 mcg. of vitamin B₁₂ a day. PCA activity was not appreciably changed by administration of vitamin B₁₂ but the B₁₂ level was significantly increased.

University of Wisconsin
Madison, W. H.

- 723 DOCTOR, V. M., and COUCH, J. R. *Bioautographic analysis of blood for growth factors active for L. leichmannii 4797* Proc. Soc. Exper. Biol. & Med. 81 222-224 Oct. 1952.

Aqueous extracts of autolyzed blood samples from various species were chromatographed on paper strips and analyzed by bioautographic procedures.

Chicks

- 724 COUCH, J. R., OLCESE, O., and GERMAN, H. L. *Vitamin B₁₂ content of chick tissues as influenced by diet*, Federation Proc. 9 355, March 1950.
- 725 COUCH, J. R., and OLCESE, O. *The vitamin B₁₂ content of chick tissues as influenced by the diet*, J. Nutrition 42 337-346, 1950.

- 726 DARBY, H. H. *Metabolism of cobalt 56 in the hen's ovary* Federation Proc. 9 356, March 1950.

"With the publication of the fact that cobalt is incorporated in vitamin B₁₂ and that B₁₂ is found in the droppings of chickens, it became of interest to study the fate of cobalt fed to hens. It had already been shown that increased growth could be obtained by injecting small amounts of B₁₂ into developing eggs. This would lead one to believe that there was a shortage of B₁₂ in the average hen's diet. Cobalt 56 was fed in pellet form to 2 hens and the eggs collected for some 3 weeks thereafter. The eggs were tested as a whole first for radioactivity and then they were separated into shell, albumen, and yolk. The amount of cobalt in each fraction was ascertained. The shell and the albumen followed a typical excretory pattern. There was a rise of radioactive cobalt in the shell and the albumen on the 4th-5th day followed by a fall to almost zero by the 10th day. The rise of radioactive cobalt in the yolk, however, was somewhat slower and although there was a high level for some 10 days, no fall of cobalt was found similar to that in the albumen and the shell. It leads one to believe that the yolk of the egg follows a different pattern of metabolism than does that of the shell and the albumen. From these experiments it seems that cobalt once laid down in the yolk is retained in these formative eggs."

Corneal Institution of Washington
Washington, D. C.

- 727 JACKSON, J. T., MACHLIN, L. J., BRANDENBURGER, E. A., KELLOGG, W. L., and DENTON, C. A. *Retention of Co 60 labeled vitamin B₁₂ in chickens*, Proc. Soc. Exper. Biol. & Med. 83 221-222, June 1953.

Authors summary "The results indicate 30% of the injected Co 60 activity is retained as 12 weeks in chicks hatched from eggs in

radiovitamin. The total B₁₂ content of the chick as measured microbiologically roughly parallels Co 60 activity until 6 weeks of age, after which there is a decided increase in the total B₁₂ content of the chicks."

U. S. Department of Agriculture
Beltsville, Md.

Rats, Africa

728. LEWIS, U. J., REGISTER, U. D., and ELVEH JEI, C. A.: *Vitamin B₁₂ content of various organs and tissues of the rat*, Proc. Soc. Exper. Biol. & Med. 71: 509-511, July 1949

Assays are reported showing no appreciable amount of vitamin B₁₂ in the liver, heart, small intestine, or muscle of rats kept on a basal corn-soybean diet for six weeks. The kidney contained 0.04 microgram of vitamin B₁₂ per gram. Addition of vitamin B₁₂ to the diet increased the amount of B₁₂ in all the organs and tissues tested. The amounts, in micrograms per gram, were as follows: kidney 0.17; liver 0.06; heart, 0.07; intestine, 0.04; spleen, no measurable amount; muscle, trace.

729. CUTHBERTSON W. F. J., FREE, A. A., and THORNTON D. M.: *Distribution of radioactive cobalt in the rat*, Brit. J. Nutrition 4: 42-48, 1950.

Cobalt is preferentially concentrated in the liver, kidney, spleen (and pancreas).

730. RICHARDSON L. R., WITTEN P. W., and COUCH, J. R.: *Diet of mother and vitamin B₁₂ content of tissues of infant rats*, Proc. Soc. Exper. Biol. & Med. 76: 265-267 Feb. 1951.

Experiments were conducted to determine the effect of various vitamin B₁₂ supplements added to the mothers' diet on the B₁₂ content of the liver, spleen, heart, and kidney of 28 day old rats. The mothers were fed the B₁₂ products from 28 days of age until at least four litters were produced.

Group	Vitamin B ₁₂ (mcg./Kg. food)	Average mcg. B ₁₂ /100 Gm. of Tissues of Young			
		Liver	Spleen	Heart	Kidney
1	None	6.1	7.7	1.7	12.8
2	36 (w/ 100.3)	8.7	13.4	4.3	16.6
3	6 (w/ succinate)	8.4	13.7	3.9	13.8
4	36 (Liver "L")	12.9	12.9	3.4	16.0

These results show that when Liver "L" was added to the basal ration the amount of vitamin B₁₂ in the liver, spleen and heart approximately doubled. The diet of the mother had no significant effect on the B₁₂ content of the kidney. The kidney and spleen contained slightly more vitamin B₁₂ than the liver. In comparison to other tissues, the heart contained a relatively small amount of B₁₂.

Agricultural and Mechanical College of Texas
College Station, Texas

731. CHOW B. F., ROSENBLUM, C., SILBER, R. H., WOODBURY D. T., YAMAMOTO R., and LANG C.: *Oral administration of vitamin B₁₂ containing cobalt⁶⁰ to rats*, Proc. Soc. Exper. Biol. & Med. 76: 393-395, Feb. 1951.

The fate of orally administered vitamin B₁₂ in the body was studied by means of a vitamin B₁₂ preparation made radioactive by cobalt⁶⁰. Daily collections of urine and feces were made for two days before and three or four days after the administration to rats of a single oral dose of 0.89 mg. of radioactive B₁₂. The animals were sacrificed on the fifth day and the kidneys, liver, spleen, brain, testes, and hearts removed. It was found that the amount of vitamin B₁₂ excreted in the urine was very low compared to the large doses administered—never more than 5%. The greatest elimination occurred within the first 24 hours. The vitamin B₁₂ content of the feces was high; in one instance, equivalent to 65% of the amount added to the diet. In the kidneys and liver, radioactivity was equivalent to 0.5% of administered B₁₂. Radioactivity of the blood, though small, indicated the passage of B₁₂ through the blood stream. The quantities listed for the remaining organs were doubtful. The dose of 0.89 mg. of vitamin B₁₂ is far in excess of physiologic requirements, and, therefore, its presence in the urine, even in low values, proves that intestinal absorption takes place upon oral administration.

Johns Hopkins University
Baltimore, Md.
Research Research Laboratories, and
Health Institute for Therapeutic Research
Baltimore 55

732. CHOW B. F., BARROWS, L., and LING, C. T.: *The distribution of radioactivity in the organs of the fetus or of young rats borne by mothers injected with vitamin B₁₂ containing Co⁶⁰*, Arch. Biochem. & Biophysics 34: 151-157 Nov. 1951.

Vitamin B₁₂ is apparently transferred placentally. After administration of vitamin B₁₂ containing Co⁶⁰ to pregnant rats, the vitamin was found in small amounts in liver and kidneys, in even lesser amounts in intestines and in insignificant amounts in spleen and heart of mother rats. The carcasses of the newborn contained large amounts.

733. ELLINGSON R. C., MUELLER, A. J., COX, W. M., JR., and CHOW B. F.: *Appearance of radioactivity in milk of rats after oral and parenteral administration of vitamin B₁₂ containing cobalt⁶⁰*, Federation Proc. 11: 441, March 1952.

"With the availability of vitamin B₁₂ containing cobalt⁶⁰ and the development of a satisfactory method of milking small animals it became possible to determine the appearance of radioactivity in milk of rats after oral and parenteral administration of vitamin B₁₂. Two groups of lactating rats were given 300 or 500 µl. of vitamin B₁₂ orally and a 3rd group 800 µl. subcutaneously. The rats were milked at various intervals (2-16 hours) thereafter and the radioactivity of the milk measured. Each of the 54 rats was milked only once; the volume was 0.5-4.0 cc./rat. No radioactivity was found in milk of 28 of the animals receiving vitamin B₁₂ orally and only traces in milk of the other 10. Large amounts of radioactivity were found in milk of 15 rats receiving the vitamin subcutaneously; a trace was found in the milk of one. There was no correlation between the quantity of radioactivity in the milk and the interval between administration of the vitamin and milking of the rats. The mothers were returned to their young and subse-

quently 1 rat from each litter was killed. The radioactivity of the kidneys, liver and carcass was determined. None could be detected in these tissues. Assuming radioactivity of the milk to be a measure of vitamin B₁₂ at least 3.5 times as much B₁₂ is secreted/cc. of milk by rats receiving the vitamin subcutaneously than by those receiving it orally."

Med Johnson Research Laboratories
Evanston, Ind.

Johns Hopkins University
Baltimore, Md.

- 734 HARTE, R. A., CHOW B. F., and BARROWS, L.: *Disappearance of radioactivity from various organs of normal adult rats following subcutaneous injection of tagged vitamin B₁₂*, Federation Proc. 11 446, March 1952.

"Following the earlier demonstration that subcutaneously injected radioactive (Co⁶⁰) vitamin B₁₂ led to retention of radioactivity in the tissues of various organs (kidney liver muscle, etc.) even after 2 weeks, estimation of the rate of disappearance of such concentrations of radioactivity becomes of interest. Thirty healthy adult rats, both males and females, from the stock colony were injected, subcutaneously with a single dose of 2 µg. of tagged vitamin B₁₂ (specific activity 60 µc./mg.) The animals were kept in individual metabolism cages. Urine specimens were collected after 1 7 and 14 days and assayed for vitamin B₁₂ by the microorganism adsorption technique of Davis and Chow. Groups of 5 animals (2 males and 3 females) were sacrificed at intervals and various organs and tissues carefully dissected out. Following acid digestion, radioactivity of the cobalt salts was determined. The findings indicate that after the first day more radioactivity is localized in the kidneys than elsewhere, but this amount tended to decrease sharply with time. Lesser absolute amounts but much higher concentrations of activity were found in the pancreas. The amounts of radioactivity in liver, pancreas, muscle and spleen remained essentially constant or even increased with time. Vitamin B₁₂ in liver is apparently associated with the protein moiety since the total amount of radioactivity remains constant but the concentration rises when the

liver glycogen is mobilized as a result of caloric deprivation."

Sherry and Bohman, Inc.
West Point, Pa.
Johns Hopkins University
Baltimore, Md.

- 735 HARTE, R. A., CHOW B. F., and BARROWS, L.: *Storage and elimination of vitamin B₁₂ in the rat*, J Nutrition 49 669-678, April 10, 1953

Authors summary: "Data are presented on the storage of radioactivity in selected target organs following administration to rats of Co⁶⁰ containing vitamin B₁₂. Noteworthy is the persistence, up to three months, of significant storage in the pancreas.

"The effects on storage in selected organs of flooding of the host with the vitamin have been studied. In this instance some evidence suggestive of flushing out in liver and pancreas has been found, but the storage of radioactivity in the kidney appears to be unaffected.

"The state of saturation of the host has been shown to play a role in the extent of storage of a tagged dose in the liver, pancreas and kidneys. In the first two organs, storage is less in the saturated animal than in the kidneys, greater storage is seen."

Sherry and Bohman, Inc.
West Point, Pa.
Johns Hopkins University
Baltimore, Md.

736. SWENDSEID M. E., BETHELL, F. H., and ACKERMANN W. W.: *The intracellular distribution of vitamin B₁₂ and folic acid in mouse liver*, J Biol. Chem. 190 791-798, June 1951.

The folic acid activity of mouse liver is due to its folic acid content. Under standard dietary conditions, folic acid is absent from the liver or present in very small amounts. The intracellular distribution patterns of vitamin B₁₂ and folic acid in liver have been determined. Vitamin B₁₂ is concentrated in the mitochondria, whereas folic acid appears to be equally distributed in the particulate and supernatant fractions.

University of Michigan
Ann Arbor, Mich.

- 737 MOVITT E. R. *Megaloblastic erythropoiesis in patients with cirrhosis of the liver* Blood 5 468-477, May 1950.

Case reports are given of 3 patients with cirrhosis of the liver, macrocytic anemia, and megaloblastic proliferation in the bone marrow. These patients gave histories indicating grossly deficient diets for many years. In each case free hydrochloric acid was present in the gastric juice, which was thought to exclude the possibility of co-existing Addisonian pernicious anemia. Vitamin B₁₂ was used in the treatment of one patient; remission followed a single dose of 150 mcg.

Formerly Admetra House Hospital, Oakland, Calif.

738. STEIGMANN F. *Advances in the management of jaundices: experiences with 500 jaundiced patients* J.A.M.A. 144 1076-1081 Nov 25, 1950.

In the discussion of dietary treatment it is stated that patients with jaundice should receive supplementary vitamins in addition to a diet high in calories and vitamins. The plasma of jaundiced patients is low in vitamin A. Doses of 50,000 to 100,000 units of vitamin A in oil are most likely to cause a rise of the plasma vitamin A level. The recent preparation of vitamin A in aqueous solution is better absorbed and more economical because a dose with about one-half the unit value of the oily solution will similarly raise the vitamin A level of the plasma. Patients who cannot take anything by mouth should be given the vitamin A parenterally, preferably in the form of vitamin A palmitate in aqueous dispersion. Jaundiced patients should receive vitamin K when their prothrombin levels are low and they also require large doses of vitamin C (500 to 1,000 mg. daily) because it has a favorable effect on carbohydrate and protein metabolism and prevents fat deposition. The B complex vitamins are very important to jaundiced patients. Natural B complex is preferable to individual synthetic B complex factors because the former contains unknown factors which may be required. Vitamin B₁₂ should be given in large doses (30 mcg. daily or more) to patients with liver disease.

University of Illinois College of Medicine, Chicago, Ill.

- 739 CAMPBELL, R. E., and PRUITT F. W. *Vitamin B₁₂ in the treatment of viral hepatitis: a preliminary report*, Am. J. M. Sc. 224 252-262, Sept. 1952.

Authors' summary: "Three groups of 100 patients each, who had acute viral hepatitis, have been compared with respect to clinical course, duration of illness and incidence of relapse. The groups were comparable as regards age, race, duration of illness and degree of icterus. Group I received the conventional treatment consisting of a high-protein, high-carbohydrate, moderate-fat diet, bed rest, multivitamins and brewer's yeast. Group II received the same diet and bed rest without added vitamins or brewer's yeast. Group III received the same treatment as Group II but with the addition of 30 mcg. of vitamin B₁₂ by mouth daily for the first 5 days of hospitalization. The following results were observed:

"1. The patients who received vitamin B₁₂ had a more rapid return to normal appetite and liver size than did the patients in the other two groups.

"2. The total serum bilirubin values of all patients in the vitamin B₁₂ group were normal 10th week of illness, whereas 18 weeks were for Group II, and 24 weeks by Group I.

"3. The mean duration of illness, calculated date of the onset of the first symptom to the date function tests became normal and remained so, days for the patients who received vitamin B₁₂ was 45 for those who received the prescribed diet and 56.7 days for those who received conventional regimen for hepatitis.

"4. There were 5 instances of relapse in group, 4 such instances in the group receiving bed rest and only 2 among the patients given vitamin B₁₂.

"5. Experimental work links vitamin B₁₂ protein metabolism and, hence, liver repair; a lipotropic agent is further evidence of its role in liver diseases. The relationship of vitamin B₁₂ acid in this process is considered.

"6. Possible objections to the validity of obtained herein are raised, but it is concluded results are sufficiently suggestive as to warrant investigation."

Rich Jackson Hospital (Hepatitis Center) and Office of Medical Consultants, C.F.C., F.C.C. San Francisco, Calif.

740. DROUET P. L., WOLFF KARLIN and G. *Vitamin B₁₂* J.A.M.A. 146 493, Jan (in Foreign Letters, Paris)

Studies were made of vitamin B₁₂ levels and animal liver in vitro. In the ox the levels ran 0.5 to 1.5 mcg. per Gm. of liver parenchyma. Tissue from human corpses revealed a B₁₂ content to 0.7 mcg. per Gm. of body tissue. In 8 patients with various diseases, liver biopsies were done and content of the hepatic tissue varied from 0.47 mcg. Vitamin B₁₂ hepatic levels were studied in patients, before treatment, a considerable fall in hepatic B₁₂ levels (0.023, 0.037, 0.041, 0.071 mcg.) was noted after treatment with a liver extract with vitamin B₁₂ in a dosage of 30 to 50 mcg. A considerable rise in the hepatic vitamin B₁₂ occurred, the increased levels being two to five times than the pretreatment levels.

- 741 GIRDWOOD R. H. *Megaloblastic anemia* Lancet 2 1063, Dec. 8, 1951 (in Soc. P.

It was reported that by bacteriological methods had been found that normal liver tissue (obtained from a patient with megaloblastic anemia) contained much folic acid, but no folic acid in a case of pernicious anemia where the patient died from another cause after receiving only a small amount of vitamin B₁₂. Folic acid and folic acid were in equal proportions. No vitamin B₁₂ had been given to the tissues of an untreated pernicious anemia patient who had died; there was no evidence of an inhibitory effect since added vitamin B₁₂ could be recovered.

ANIMAL STUDIES

REGENERATION STORAGE, NECROSIS

742. DENTON R. W., and IVY A. C. *Effect of feeding liver on the rate of regeneration of the liver in partially hepatectomized rats* Am. J. Physiol. 152 460-464, Feb. 1948.

"Liver tissue, either because it contains a high quality protein or some as yet undetermined growth-promoting factor other than thiamine or the anti-pernicious anemia factor facilitates the rate of regeneration of the liver in the partially hepatectomized rat."

743. STERN J. R., TAYLOR, M. W., and RUSSELL, W. C. *Relation of vitamin B₁₂ to liver basophilia*, Proc. Soc. Exper. Biol. & Med. 70 551-552, March 1949

Growth of weanling white rats was stimulated by inclusion of vitamin B₁₂ in the diet at a level of 440 mcg./Kg. Whole dried liver also stimulated growth. Rats that received neither supplement grew poorly and showed little or no liver basophilia, whereas those receiving B₁₂ or liver grew well and showed considerable cytoplasmic basophilia in their liver cells.

Authors Summary
J. R. Stern, et al.

744. VARS, H. M., KARN G. M., and FERGUSON, C. C. *Vitamin B₁₂ and liver protein regeneration*, Federation Proc. 9 373-374 March 1950.

"Adult male rats reared on a Purina diet were fed a nonprotein diet for 2 weeks and subjected to a 70% partial hepatectomy. During a 2 week postoperative period suitable groups were fed diets containing no protein, 10 or 18% casein and 10% wheat gluten, with and without the substances or intraperitoneal injection of a total of 4-10g of Cobalamin. There were no essential differences between treated and untreated groups in the N-balance, food intake, weight restoration and liver protein regeneration. A similar experiment was conducted with rats that had been fed a soya protein diet, free of animal protein factor for a period of 8-13 months. After 2 weeks on a non-protein diet and partial hepatectomy they were fed the same soya diet for 14 days, with and without injection of Cobalamin. The ad libitum-fed B₁₂ treated rats regained more in body weight and regenerated as much liver protein (slightly less positive N-balance) than the untreated controls. The B₁₂ treated controls, pair fed to the untreated controls regained the same amount of body weight but regenerated less liver protein and had a smaller positive N-balance. Under experimental conditions employed vitamin B₁₂ has evidenced little or no stimulatory effect in facilitating liver protein regeneration."

University of Pennsylvania School of Medicine
Philadelphia, Pa.

745. GYÖRGY P., and ROSE, C. S. *Effect of vitamin B₁₂ on experimental hepatic injury* Proc. Soc. Exper. Biol. & Med. 73 372-375, March 1950.

Authors summary "Vit. B₁₂ (1g daily by mouth) had no effect on the development of massive hepatic necrosis in rats fed experimental rations with yeast as sole source of protein.

"Vit. B₁₂ (0.5g daily by mouth) has shown significant lipotropic activity in rats fed a low protein-low fat ration. Under the experimental conditions chosen methionine was more effective than vitamin B₁₂ in preventing fat deposition in the liver. A low protein-high fat diet seemed to interfere with the lipotropic effect of B₁₂."

University of Pennsylvania School of Medicine
Philadelphia, Pa.

746. DIETRICH, L. S., MONSON W. J., and ELVEHJEM, C. A. *Observations on a relationship between vitamin B₁₂, folic acid and the citrovorum factor* Proc. Soc. Exper. Biol. & Med. 77 93-96, May 1951

Studies of the relationship between vitamin B₁₂, folic acid (PCA) and the Leuconostoc citrovorum factor (LCF) are presented. In chicks deficient in PCA and vitamin B₁₂, the intramuscular injection of low levels of B₁₂ (0.1 or 0.3 mcg. per day) increased the PCA stored in the liver but higher levels (0.5 or 1.0 mcg.) suppressed liver storage of PCA. Oral administration of 0.3 mcg. of vitamin B₁₂ gave results similar to those obtained by intramuscular injection of the same amount.

When the vitamin B₁₂-deficient chicks received ample PCA, the oral administration of 0.5 mcg. of vitamin B₁₂ per day produced a marked increase of both LCF and PCA levels in the liver while parenteral administration depressed the storage of PCA without changing that of LCF. Oral administration produced slightly lower levels of liver storage of vitamin B₁₂ than did parenteral administration of the vitamin.

Further experiments in chicks deficient in both PCA and vitamin B₁₂ showed similar levels of this vitamin in the liver regardless of the route of administration. The liver level of PCA was lowered while that of LCF remained unchanged. Studies of liver slices from chicks given B₁₂ by injection showed decrease in the amount of PCA converted to LCF; this inhibition was overcome by the addition of ascorbic acid to the flask in which the determination was made. The amount of PCA converted to LCF was shown, with or without the in vitro use of ascorbic acid, to be significantly increased in chicks given vitamin B₁₂ orally.

These data demonstrate that vitamin B₁₂ plays some role in the conversion of PCA to LCF but whether its action is a specific vitamin action or an indirect action is unknown. Various theories are briefly discussed.

University of Wisconsin
Madison, Wis.

747. GYÖRGY P., STOKES J. JR., GOLDBLATT H., and POPPER, H. *Antimicrobial agents in the prevention of dietary hepatic injury (necrosis, cirrhosis) in rats*, J. Exper. Med. 93 513-522, June 1951

Authors summary: "The effect of various antimicrobial agents, such as aureomycin, terramycin, streptomycin, chloromycetin, penicillin, polymyxin, and sulfa guanine on the development of massive dietary necrosis of the liver in rats has been studied. Delay in the production of hepatic necrosis was obtained from aureomycin and, to a lesser extent, from terramycin and

Indication of temporary protection was shown by sulfaguanidine, whereas chloromycetin, polymyxin, and penicillin were not protective.

"B₁₂, added alone, or in combination with aureomycin, to the basal experimental diet had no influence on the development of hepatic necrosis. A combination of pectin with streptomycin enhanced the protective effect of the antibiotic.

"All the antimicrobial agents tested, without relation to their effect on hepatic necrosis, produced temporary stimulation of growth in the experimental animals.

"The beneficial effect of aureomycin was not limited to the delay of hepatic necrosis but manifested itself also in the prevention of hepatic cirrhosis in rats fed a low protein (casein)-high fat diet. In contrast to control animals showing the usual combination of cirrhosis and renal changes, the rats receiving supplements of aureomycin were free of both cirrhosis and renal changes. The rats receiving aureomycin took more food in and gained weight.

"No microscopic alterations were seen in the pancreas of the control rats with cirrhosis.

"In both groups of experiments (necrosis and cirrhosis) the antimicrobial agents, with the exception of penicillin, were given mixed with the food. Their possible effect on the intestinal flora is discussed."

748. ABELL, M. R., and BEVERIDGE, J. M. R. *Studies on hepatic necrosis induced by dietary means IV Conditions affecting the production and prevention of massive liver necrosis*, A.M.A. Arch. Path. 52 428-440, Nov 1951.

Authors summary in part "In an attempt to elucidate further the factors involved in the production of massive liver necrosis in rats fed diets low in methionine, cystine and alpha-tocopherol, experimental conditions have been varied with regard to initial weight and sex of the test animal and with regard to various dietary supplements.

"The inclusion of cod liver oil greatly increased the necrogenic quality of the basal diet.

"Of the lipotropic and antilipotropic substances tested, choline, inositol, cholesterol, and cystine, only the last had any effect on the development of liver necrosis. This substance displayed its well-known prophylactic action.

"In confirmation of previous reports, alpha-tocopherol, like cystine, prevented the development of hepatic damage. A similar but definite effect was noted for synthetic vitamin K (menadiolone).

"Sulfaguanidine and aureomycin both caused definite amelioration of the necrogenic property of the basal diet.

"With the exception of vitamin B₁₂ alterations in the vitamin supplement had no effect. In the case of B₁₂ there appeared to be a slight increase in the time required for death due to liver necrosis, but this difference could not be shown to be of statistical significance."

University of Western Ontario
London, Ontario, Canada

749. SCHEID H. E., ANDREWS M. M., and SCHWEI GERT B. S. *Liver storage of vitamin B₁₂ by the rat*, Proc. Soc. Exper. Biol. & Med. 78 558-560, Nov 1951

Storage of vitamin B₁₂ in the liver of the rat was studied by determining the vitamin B₁₂ potency for the growth of *Lactobacillus leichmannii* and *Engelmannia gracilis* of livers from rats fed different levels of vitamin B₁₂. The vitamin B₁₂ potency was shown to increase as the level in the diet of the rats was increased. The values were only 1/10 to 1/20 those in beef liver however. Unlike that of beef liver the vitamin B₁₂ potency of rat liver was not reduced appreciably by alkali treatment. The vitamin B₁₂ potency remaining in rat livers after alkali treatment may be due to the presence of alkali stable forms of vitamin B₁₂ as well as to desoxyribosides. The weight of the liver in proportion to body weight was greater in rats on a vitamin B₁₂-deficient diet than in those on the optimal diet.

American Meat Institute Foundation, and
The University of Chicago
Chicago, Ill.

750. DUMM, M. E., and RALLI E. P. *Effects of diet, partial hepatectomy and growth promoting factors on the composition of the rat liver* Federation Proc. 11 440-441 March 1952.

"Adult rats were maintained on a 9% protein, 38% fat diet for 42 days and were then partially hepatectomized. The liver removed was analyzed for lipids, nitrogen and solids. After operation, the rats received the same diet without any supplement or with injections of liver extract or vitamin B₁₂. Other rats were transferred after operation to 16 or 26% protein (9% fat) diets. The animals were sacrificed 6-15 days after operation and the analyses repeated. In the rats continued on the 9% protein diet with no supplement liver lipids increased and nitrogen decreased from operation to sacrifice. Injections of liver extract or increasing the protein in the diet had some lipotropic effect and also increased liver nitrogen. Vitamin B₁₂ had a pronounced and consistent lipotropic effect associated with an increase in liver nitrogen. Microscopically the livers of rats on the low protein diet with no supplement showed increased lipid deposition at sacrifice. Rats given liver extract or the higher protein diets showed decreased liver fat and evidences of cellular regeneration. Rats receiving vitamin B₁₂ showed striking evidences of cellular regeneration."

New York University-Belmont Medical Center
New York, N. Y.

LIPOTROPISM

751. HALL, C. A., and DRILL, V. A. *Lipotropic effect of liver extract on dietary hepatic injury in rats*, Proc. Soc. Exper. Biol. & Med. 69 3, Oct. 1948.

Fatty change and fibrosis occurring in the livers of rats fed on a diet high in fat but not low in protein were prevented by supplementing the diet with choline or crude liver extract. The lipotropic effect of the liver extract is not due to an increase in protein intake, nor to the small amount of choline present in the liver extract.

752. RODNEY G., SWENDSEID M. E., and SWANSON L. *The role of pteroylglutamic acid in tyrosine oxidation by rat liver tissue* J Biol. Chem. 179 19-21 May 1949

Liver tissue from rats with a succinylsulfathiazole-induced deficiency in pteroylglutamic acid (PGA) shows a decreased oxidation of tyrosine compared with normal liver tissue. The oxidation of tyrosine can be partially restored by the addition in vitro of PGA, but not by PGA conjugate or by liver extract. Liver tissue from rats with PGA deficiency induced by feeding 4-amino-PGA also shows decreased tyrosine oxidation. In vitro this effect cannot be reversed by the addition of PGA, liver extract, or vitamin B₁₂ concentrate but in vivo it can be reversed by the administration of either PGA or liver extract. The addition of 4-amino-PGA in vitro to normal rat liver does not affect the rate of tyrosine oxidation.

Purdue, Davis and Company
Bloomington, Mich.

753. DRILL, V. A., and McCORMICK, H. M. *Lipotropic effects of vitamin B₁₂ concentrate* Proc. Soc. Exper. Biol. & Med. 72 388-390, Nov 1949

Injection of vitamin B₁₂ concentrate into rats on a high-fat diet exerted a marked lipotropic effect which was not due to the small amount of choline present in the concentrate.

754. SCHAEFER, A. E., SALMON W. D., and STRENGTH, D. R.: *Role of vitamin B₁₂ and methyl donors in lipotropism and transmethylation in the rat and chick*, Federation Proc. 9 369-370, March 1950.

"The relation of vitamin B₁₂ to the quantitative effectiveness of monomethylaminoethanol, dimethylaminoethanol, betaine, DL-methionine and choline as methyl donors for the prevention of renal damage and fatty livers in rats and for growth and perosis-prevention in chicks was studied. The choline requirement of weanling rats for protection against fatty livers was reduced from 0.16% to 0.10% when the diet was supplemented with vitamin B₁₂ and folic acid. On the basis of methyl content monomethylaminoethanol and betaine were considerably less active for growth and prevention of renal damage and fatty livers in the rat than dimethylaminoethanol or choline when vitamin B₁₂ and folic acid were omitted from the diet. In the presence of vitamin B₁₂ all the above compounds were equivalent to choline on a methyl basis. Three mols of betaine were required to replace 1 mol of choline. For the chick, betaine, DL-methionine or monomethylaminoethanol, even in the presence of vitamin B₁₂, could not replace choline for growth and protection against perosis. Monomethylaminoethanol and either DL-methionine or betaine could replace choline on a methyl basis only when supplemented with vitamin B₁₂. Vitamin B₁₂ appears to function in methylation processes."

Michigan Polytechnic Institute
Ann Arbor, Mich.

755. McCORMICK, H. M., and DRILL, V. A.: *Lipotropic effects of liver extract, vitamin B₁₂, and choline*, Proc. Soc. Exper. Biol. & Med. 74 626-630 July 1950.

In experiments with rats on a high fat diet, it was found that 1 cc. of crude liver extract given subcutaneous

ously three times a week was approximately the minimum effective lipotropic dose. Given orally this amount had slightly less lipotropic effect, and lower subcutaneous doses were less effective.

Since both the liver extract and the vitamin B₁₂ concentrates previously reported to have lipotropic action (Drill and McCormick, Proc. Soc. Exper. Biol. & Med. 72 388, 1949 [Abstr. 753]) contain small amounts of choline, folic acid, and inositol, a combination of these substances was tested, but was found to be ineffective.

Crystalline B₁₂, even in doses of 2.5 mg., six times a week subcutaneously was ineffective, and combinations of B₁₂ and choline did not prevent fatty changes in the liver although animals receiving this combination gained more weight than animals receiving choline alone.

The authors conclude that the lipotropic activity of liver extract or of vitamin B₁₂ concentrate must be due to factors other than those discussed above. The possible effects of combinations of crystalline vitamin B₁₂ and suboptimal amounts of folic acid, with or without choline, are being studied.

Ypsilanti University College of Medicine
Detroit, Mich.

756. KELLEY B., TOTTER, J. R., and DAY P. L.: *The lipotropic effect of folic acid on rats receiving various purified diets*, J Biol. Chem. 187 529-535, Dec. 1950.

The addition of vitamin B₁₂ to the diets used in these experiments brought about little alteration in any of the fat fractions in the liver. Since the diets used contained 18 per cent casein, it is probable that the requisite amount of vitamin B₁₂ was thus supplied, so that additional amounts of this vitamin might be expected to have no appreciable effect.

University of Arkansas
Little Rock, Ark.

757. LUCAS, C. C., RIDOUT J. H., PATTERSON J. M., and BEST C. H. *Lipotropic properties of vitamin B₁₂*, Federation Proc. 10 218, March 1951.

"The lipotropic properties of vitamin B₁₂ have been assessed in weanling male rats of the Wistar strain. Effects upon rate of growth, deposition of liver lipids and development of renal lesions have been followed for periods up to 17 days. The interrelationships among choline chloride, vitamin B₁₂ and folic acid have been examined by supplementing a hypolipotropic basal diet with each one separately at several levels, and in various combinations. Effects of the vitamins on food intake were controlled by conducting both *ad libitum* and paired feeding experiments. Vitamin B₁₂ had no effect on the liver lipids in these experiments."

University of Toronto
Toronto, Ontario, Canada

758. STRENGTH, D. R., SCHAEFER, E. A., and SALMON W. D.: *The relation of vitamin B₁₂ and folic acid to the utilization of choline and its precursors for lipotropism and renal protection in rats* J Nutrition 45 329-343, Nov 10, 1951

Vitamin B₁₂ and folic acid were ineffective as lipotropic substances when fed singly to rats receiving a fixed basal diet supplemented with 0.1 per cent ch

chloride. A combination of B_{12} and folacin reduced liver fat from an average of 43 to 18 per cent. Liver fat of the rats fed the basal ration fortified with 0.08 and 0.12% choline chloride and also with B_{12} concentrate and folacin was equal to that of rats receiving 0.12 and 0.16% choline chloride respectively without supplements of B_{12} and folacin. Choline chloride at an 0.20% level was approximately as effective for the maintenance of normal liver fat as was the same level of choline chloride plus B_{12} and folacin. The lipotropic activity of vitamin B_{12} and folacin appeared to consist of a choline-sparing effect, which was manifest at subprotective levels of choline. Vitamin B_{12} and folacin added to the basal ration supplemented with varying levels of betaine or dl-methionine exerted a marked sparing effect on the requirement of these substances for maximum growth, prevention of renal damage, and lipotropism.

The addition of betaine HCl or dl-methionine at an 0.08% choline chloride equivalent level, without B_{12} and folacin resulted in a 100% incidence of renal damage as compared to 13% for choline chloride. Three moles of betaine HCl or dl-methionine, when added to the diet supplemented with B_{12} and folacin were as effective as one mole of choline for protection against renal damage and lipotropism.

Neither dimethylaminoethanol nor methylaminoethanol fed to rats at varying levels was equal to choline for growth promotion and maintenance of normal liver fat. Increasing the level of methylaminoethanol or dl-methylaminoethanol in the absence of B_{12} and folacin resulted in a depression of body weight gain. When these compounds were fed at a choline chloride equivalent level of 0.16% (without B_{12} and folacin) average liver fat values were 36 and 26% respectively as compared with 17% when choline was fed. The addition of B_{12} and folacin reduced liver fat. Dimethylaminoethanol was considerably more active than methylaminoethanol. Trimethylcholine was not capable of replacing choline as a lipotropic agent for weanling rats.

With a more highly purified diet, in which the casein and peanut meal were thoroughly extracted with methanol, supplementation with betaine or dl-methionine at a choline equivalent level of 0.08% plus vitamin B_{12} and folacin, resulted in 100 and 50% renal damage, respectively. When vitamin B_{12} and folacin were added to the highly purified diet supplemented with methylaminoethanol and dl-methionine at a choline equivalence of 0.12% the lipotropic activity of this combination was equal to that of choline. When B_{12} and folacin were not included, the lipotropic activity was not equal to that of choline. The addition of dl-methionine, or betaine HCl, or dl-methionine plus aminoethanol at a choline chloride equivalent level of 0.08% resulted in 100% renal damage in rats on the more purified diet. Supplementation of this ration with B_{12} and folacin, and either dl-methionine or betaine HCl, resulted in 30 and 100% renal damage, respectively. Further addition of aminoethanol to these diets gave complete protection against renal damage and supported maximum growth.

Results obtained in these experiments indicate that B_{12} and folacin appear to be essential for the maximum utilization of methionine or betaine for the biological

synthesis of choline from aminoethanol or methylaminoethanol.

*Alabama Polytechnic Institute
Tuscaloosa, Ala.*

- 759 FISCHER, M. A., and HALL, G. D.: *Relation of dietary protein to signs of choline deficiency—effect of supplements of vitamin B_{12} and folic acid*, Federation Proc. 11 211-212, March 1952.

"By the tenth day 75% of the rats fed a diet containing 15% casein showed signs of acute choline deficiency. The kidneys were enlarged and hemorrhagic, the serum non-protein nitrogen had risen to 70 mg.% and the livers contained an excess quantity of fat. Addition of B_{12} and folic acid to this diet reduced liver fat and prevented kidney damage. Both control and supplemented groups fed a 9% casein ration exhibited poor growth and neither group showed signs of choline deficiency how ever the lipotropic effect of the supplement was evident. When animals were fed a 24% casein diet the vitamin supplements had no apparent effect on growth response, liver weight and fat content, or kidney weight. The serum NPN of both of these groups was about 80 mg.% In contrast, the supplement reduced the NPN from 26 mg.% to 20 mg.% when the animals were maintained on 9% casein. Low values were also observed when the supplemented 15% casein diet was fed. Serum albumin content was low in animals fed the 9% casein diet. B_{12} and folic acid additions to the diet slightly raised the level. The supplements had least effect on serum albumin of rats fed the 24% casein diet. Changing the protein content or adding the vitamins to the diets caused some changes in the serum globulins."

*University of Pittsburgh School of Medicine
Pittsburgh, Pa.*

760. BEST, C. H., HARTROFT, W. S., and SELLERS, E. A.: *The protection of the liver by dietary factor* Gastroenterology 20 875-884, March 1952.

This general descriptive (primarily histologic) article summarizes present knowledge on the effects of choline deficiency on the liver of experimental animals. Choline deficiency causes an intensely fatty liver and loss of liver function. Restoration of choline to the diet cures these conditions. Cirrhosis due to choline deficiency in animals can be cured by choline if the cirrhosis is not too advanced. The signs of choline deficiency are apparent within 24 hours of the beginning of the low-choline regimen and within a week all the liver cells are distended with fat. Stainable lipid first appears in the liver as small intracellular globules which coalesce to form large masses which distend the cell membrane and then rupture it so that fatty cysts are formed. Later these involve biliary and vascular channels. The stages of removal of fat from the liver upon restoration of choline to the diet are described and illustrated in detail. Intracellular fat disappears more rapidly than extracellular fat, the latter taking several months.

It is pointed out that calorie restriction has a "pseudo-lipotropic" effect. Clinicians and physiologists have tried to cure by choline administration many types of liver damage which were not caused by choline deficiency. This, in the authors' opinion is futile.

Commenting on the lipotropic effects of vitamin B₁₂ and folic acid, the authors state: "Folic acid and vitamin B₁₂ are now involved in lipotropic phenomena and the amounts of these substances present in a diet may prove to affect liver function through mechanisms not directly related to those which control anemia. Methyl groups (CH₃) are important in the supply of lipotropic agents since their addition to ethanolamine, which is usually available in the body may form choline. These vitamins, folic acid and B₁₂, by favoring the synthesis of new methyl groups, may affect the apparent choline requirement of an animal and perhaps of a patient." Present attempts to use lipotropic agents therapeutically are not based on the known action of these substances in humans, but on their effects in experimental animals. If some means could be found "to produce a safe but definite choline deficiency in human subjects, the most important single step toward integrating laboratory and clinical findings in this field would have been taken."

Fordham University
New York, N. Y.

751. TRAVERS, J. J., and CERECEDO L. R. *Inter relationship between choline and vitamin B₁₂ in the mouse*, Federation Proc. 10 396-397 March 1951

"The greater resistance of the mouse to choline deficiency and the involvement of vitamin B₁₂ in the synthesis of methyl groups and choline led to a study (microbiological) of the vitamin B₁₂ content of mouse and rat tissues. Under all conditions mouse liver contained more vitamin B₁₂ than rat liver. In each species lowest values were obtained with a sulfaximidine-iodinated casein diet which, after 5 weeks, produced values for mouse liver comparable with those of stock rats, and a 6-fold increase over those of rats similarly treated. A vegetable protein diet caused a temporary decrease in each species, followed by an increase. Even after 8 months, mouse liver contained as much vitamin B₁₂ as those of young stock mice, and more than those of young stock rats. Thus, as with choline, younger animals need an exogenous supply of vitamin B₁₂. Since this vitamin is involved in the synthesis of methyl groups and the transmethylation process, it appears that the resistance of the mouse to choline deficiency can be related, in part, to its vitamin B₁₂ content. The data are also in line with our inability to produce in mice, in contrast to what has been observed in rats, tumors by choline deficiency with the recent report on the procarcinogenic effect of vitamin B₁₂ in rats and with the greater incidence of spontaneous tumors in mice."

Fordham University
New York, N. Y.

762. TRAVERS, J. J., and CERECEDO L. R. *Interrelationships between choline and vitamin B₁₂ in the rat and in the mouse*, Federation Proc. 11 457 March 1952.

"Rapid and uniform depletion of the vitamin B₁₂ stores of the livers of rats was accomplished with a diet containing sulfaximidine and iodinated casein, but free of vitamin B₁₂ and choline. The growth of these rats was stimulated by the administration of vitamin B₁₂ and of choline. Vitamin B₁₂ was found to be without effect if choline was present in the diet at an adequate level. Under

the conditions used, choline, supplied in the diet, prevented to a large extent the depletion of the liver stores of vitamin B₁₂. The choline content of the kidneys of choline deficient rats was significantly lower than that of choline fed rats, and was not affected by the administration of vitamin B₁₂. Under similar conditions, different results were obtained with mice. The administration of choline had no effect on the vitamin B₁₂ content of the livers of mice, nor on the choline content of the kidneys of mice."

Fordham University
New York, N. Y.

763. SCHAEFER, A. E., COPELAND D. H., and SALMON W. D. *Duodenal ulcers, liver damage anemia and edema of chronic choline deficiency in dogs*, J. Nutrition 43 201-222, Feb. 1951

The effects of choline deficiency were studied in dogs. Supplementing the diet of the dogs with 0.05 per cent or less of choline resulted in death in 13 to 30 weeks. The symptoms of choline deficiency preceding death were anemia, liver dysfunction, weight loss, and edema. Hepatic function became impaired in chronically choline-deficient dogs four to eight weeks before anemia developed. Subcutaneous edema and excessive fluid in the abdominal cavity were noted in 6 of the 7 dogs that died. Vitamin B₁₂, which has a sparing action on the choline requirement of rats and chicks, also protected dogs against the effects of choline deficiency in the present experiments. A vitamin B₁₂ concentrate was used. The inclusion of a source of vitamin B₁₂ in the diet prevented the development of ascites. In dogs not receiving vitamin B₁₂, the choline requirement was 0.10 per cent or greater. Autopsy studies revealed that 6 of 7 dogs who died of chronic choline deficiency had duodenal ulcers (perforated in 2). Whether these ulcers were specifically related to choline deficiency or were a secondary result of the accompanying inanition could not be determined. The livers of all choline-deficient dogs were fatty and 6 dogs had cirrhosis.

Michigan Polytechnic Institute
Ann Arbor, Mich.

764. BURNS, M. M., and McKIBBIN J. M. *The lipotropic effect of vitamin B₁₂ in the dog*, J. Nutrition 44 487-499 Aug. 1951.

Authors summary "1. A deficiency of vitamin B₁₂ is reported in weanling puppies fed on a synthetic diet containing 19% purified casein and 9% fat. The deficiency varied in severity with different litters of animals and was evident in growth impairment, abnormalities of liver function and, in several instances, gross fatty infiltration of the liver

"2. Administration of either vitamin B₁₂ or choline produced significant improvement in liver function in 4 dogs receiving the basal diet. Two dogs receiving a choline-deficient diet were cured of their fatty livers with Vitamin B₁₂ alone.

"3. These findings suggest that the dog's requirement for methylating substances, like that of the rat and chick is dependent upon the vitamin B₁₂ content of

Swansea University
Swansea, N. Y.

Toxic and Physiologic States

RADIATION SICKNESS, INJURY

765. WARREN, S. *Atomic Energy Commission report*, J. Indiana M. A. 42: 1062-1084, Oct. 1949

In a report of the Atomic Energy Commission to Congress the importance of the bone marrow and the blood in radiation sickness is mentioned. Repeated transfusions are needed by people exposed to atomic radiation, and it is suggested that new substances, such as vitamin B₁₂, which stimulate the formation of mature blood cells, will be helpful in radiation sickness.

766. PARK, W. E. *If an atomic bomb should fall*, Canad. M. A. J. 61: 473-478, Nov. 1949

The treatment outlined for victims includes prevention of infections by administration of penicillin in daily doses of 200,000 to 1,000,000 units, preferably by mouth streptomycin if the patient develops septicemia of *Escherichia coli* infection daily administration of liver extract or vitamin B₁₂ and folic acid to promote the formation of red blood cells.

Chalk River, Ontario, Canada

767. REZNIKOFF, P., in Cornell Conference on Therapy: *Problems in treatment of the atomic casualty*, New York State J. Med. 52: 1804, July 15, 1952.

In a discussion on this subject the question of the effect of antibiotics, particularly aureomycin, on radiation sickness was raised. Dr. Reznikoff cited two studies that contribute to understanding of the mechanism of action of aureomycin. Dr. Davis has shown that *Bacterium coli* organisms can absorb or inactivate large amounts of vitamin B₁₂. Watson and Jakes have obtained good results in pernicious anemia from the combined administration of B₁₂ and aureomycin. Since B₁₂ is not ordinarily effective when given orally in this disease, it is presumed that aureomycin indirectly protected the vitamin and thus permitted it to be utilized. A similar situation may occur in radiation sickness. Aureomycin may by means of its antibiotic activity on the intestine, permit absorption of essential therapeutic substances.

Cornell Medical College, and
New York Hospital
New York, N. Y.

768. BUTLER, R. E.: *Special feeding problems in an emergency*, Pub. Health Rep. 67: 867-871, Sept. 1952.

Among the subjects discussed is the intravenous and oral use of fat emulsions in feeding burned patients. As an oral preparation, the fat emulsions have also been investigated for their effect in increasing caloric intake. Recommendations are also given for the treatment of shock in an emergency.

In the discussion of radiation injuries the author states that there is little indication that such hemopoietic agents as folic acid and vitamin B₁₂ would be effective, and that iron would seem to be useless until blood regeneration is observed.

Office of the Surgeon General
U. S. Public Health Service
Washington, D. C.

769. CORNATZER, W. E., ARTOM, C., HAF, T., and CAYER, D. *Beneficial effects of B₁₂ and folic acid on recovery from intoxication by P₃₃*, Proc. Soc. Exper. Biol. & Med. 55: 554, March 1951.

Generous amounts of folic acid, or folic vitamin B₁₂ had no significant effect on the mice injected with a dose of P₃₃ in the higher LD₅₀. When sulfasuxidine was included in the over the administration of vitamin B₁₂ and increased significantly both survival time and of survivors.

University of Michigan
The Warren College
Ann Arbor, Mich.

ANTIHYPEROTIC EFFECTS

770. NUTRITION NOTES: *The anti-hyperotoc vitamin B₁₂*, Am. J. Digest. Dis. 16: 418, 1951.

An antihyperotoc effect of vitamin B₁₂ for the possible usefulness of this vitamin in treatment of hyperthyroidism are suggested by 1) that individuals with pernicious anemia compound severe hypothyroidism require a much higher of thyroid extract when they are placed on B₁₂; 2) the finding of Enabroff that feeding liver or retortoxity in rats, and 3) the observation that amounts of vitamin B₁₂ duplicate the growth of fish solubles or injected liver extracts in a colving thyrotoxin.

771. NUTRITION NOTES: *The anti-hyperotoc vitamin B₁₂*, Am. J. Digest. Dis. 17: 25, 1952.

It has been shown that vitamin B₁₂ has a retortoc effect. For example, patients suffering pernicious anemia complicated by severe hypot may require a higher dosage of thyroid extract cases, double the amount) than they needed pretherapy. Several other examples are cited. It is that B₁₂ may eventually be useful in the treatment of hyperthyroidism. Practitioners are cautioned administration of thyroid extract is less effective than who are receiving either liver extract therapy.

772. WAYNE, E. J., MACGREGOR, A. G., and H. *Vitamin B₁₂ and thyroid function*, J. 527 Feb. 18, 1950 (in Letters to the Editor).

On the basis of tests with two male patients normal thyroid function, it is concluded that in of 100 mcg. or less, vitamin B₁₂ has no significant effect on thyroid function.

University of Sheffield, and
Sheffield Medical Centre for Radiotherapy
Sheffield, England

773. QUESTIONS AND ANSWERS: *Mod. Med.* 20: 1, 1952.

"QUESTION Does 50 µg. of vitamin B₁₂ given intramuscularly have any effect on the basal metabolic rate? Does this vitamin occasionally cause a rise in temperature?"

"ANSWER: By Consultant in Pharmacology Vitamin B₁₂ has no effect on the basal metabolic rate. An increase in body temperature at the time of injection is probably caused by the presence of undesirable pyrogens."

ANEMIAS OF TOXICITY; OTHER TOXIC STATES

774. VON BONSDORFF B.: *Etude sur la vitamine B₁₂* (Study regarding vitamin B₁₂). Semaine d. hôp. de Paris 26: 3316-3317 Sept. 2, 1950.

Treatment of pernicious anemia due to the fish tapeworm (*Diphyllobothrium latum*) consists of expulsion of the tapeworm, or of administration of liver gastric preparations, or folic acid orally or parenteral administration of liver folic acid or vitamin B₁₂. Patients with this disease have reacted in two ways to vitamin B₁₂ administered orally 5 mcg. during eight days. Some of these patients have shown no response to vitamin B₁₂ either with or without gastric juice, while in others remission is obtained even without the administration of gastric juice. Secretion of the intrinsic factor in sufficient quantity would evidently establish vitamin B₁₂ action, and absorption of this product at a high level in the digestive tract where the parasites could not endure the reaction, would follow.

Reichle, Frankfurt

775. FRANK, O., LACHNITZ V., and NEUMAYR, A.: *Influence of vitamin B₁₂ on lead porphyriaemia*. Acta haematol. 8 42-1952 (abstr. J.A.M.A. 150 1433, Dec. 6, 1952)

"Increased urinary coproporphyrin B is a constant and early sign of lead poisoning. This sign is important in the diagnosis but also presents a therapeutic problem, since it is said to be related to a number of symptoms, particularly colic and neuritic symptoms. The favorable effects produced with liver extracts and the components of the vitamin B complex induced the authors of this report to try vitamin B₁₂. They present observations on 11 patients whose occupations involved exposure to lead. They were given 50 µg. of vitamin B₁₂ twice weekly by intramuscular injection. The elimination of coproporphyrin in the urine was determined at weekly intervals. Treatment with vitamin B₁₂ normalizes porphyriaemia and prevents the excessive urinary excretion of coproporphyrin induced by mobilization of lead. This effect of vitamin B₁₂ is believed to be the result of its catalytic action on hemoglobin synthesis, especially on the ribonucleotides and of its protective action on the liver."

776. TUCZEK, H., and SAUPE, M.: *Experiences with isonicotinic acid hydrazide in tuberculosis*. Munch. med. Wchnschr. 94 1307 June 27 1952 (abstr. J.A.M.A. 150 1052, Nov. 8, 1952)

This report describes the studies in a sanatorium in which more than 1,000 tuberculosis patients were under treatment for 8 to 12 weeks. In discussing effects on various systems, it was noted that in 84 patients the sedimentation rate remained unchanged, while in 108 others, it

became normal. "About 80% of the patients showed a decrease in erythrocytes after treatment with isonicotinic acid hydrazide had been continued for some time, but the erythrocyte count became normal following treatment with vitamin B₁₂."

777. KRESBACH, E., and KNORR, G.: *Pannmyelophthisis after treatment with methylphenylethylhydantoin*. Klin. med. 7: 346- Aug. 1 1952 (abstr. J.A.M.A. 150 1349 Nov. 29 1952)

"The patient was a young woman, aged 19 who had had epileptic attacks since the age of 14. For some time she had been treated with phenobarbital, but then the medication had been changed to methylphenylethylhydantoin. After many months of this treatment she had become pale, and severe anemia had been detected for which several blood transfusions had been given. At the present time the results of sternal puncture and of blood examinations indicated severe pancytopenia. In addition to blood transfusions and hemostatic drugs, large doses of vitamins C, B₂ and K were given, and attempts were made to stimulate hematopoiesis by vitamin B₁₂ and B₁₂ folic acid, and large doses of iron. Under the influence of these measures, the cutaneous and mucosal hemorrhages were temporarily arrested, but the septic fever remained unchanged. Later mucosal hemorrhages recurred, coma developed, and the patient died. An autopsy was performed, and it was assumed that the hydantoin preparation has produced toxic destruction of the bone marrow."

778. STEIN, H., and WEISSBECKER, L.: *Symptomatic pernicious anemia in chronic poisoning with chromium*. Zentralbl. Arbeitsmed. u. Arbeitsschutz, Darmstadt 3 42 March 1953 (abstr. J.A.M.A. 152 1575, Aug. 15, 1953)

"The occurrence of hyperchromic anemia, leukopenia, reduced number of reticulocytes (0.2%) Hunter's glossitis, histamine-refractory gastric anacidity and paresthesia in a workman is reported who had been employed for 25 years in a tannery. The patient had cachexia and laet-like surface defects in the oral cavity and the esophagus, which, in the presence of large amounts of chromium in the saliva and in the urine, completed the characteristic picture of chronic chromium poisoning. The classic symptoms of pernicious anemia prompted the author to give the patient vitamin B₁₂ and/or pteroylglutamic (folic) acid orally or parenterally but the patient did not respond to this treatment. He then was given continued blood transfusions. Six months after the last blood transfusion the blood counts had become normal. The ulcers in the mouth and in the esophagus were cicatrized and the amounts of chromium in the urine and in the saliva were considerably reduced, but the cachexia and the atrophy of the lingual papillae remained unchanged. There are two possible explanations for the failure of vitamin B₁₂ therapy in this patient. The synthesis of the thymonucleic acid from thymidine may have been disturbed directly by the chromium, or vitamin B₁₂ may have been inactivated by the chromium as a result of its complex affinity."

779. MAURIZIO E.: *Vitamin B₁₂ in medical therapy of toxemia of pregnancy and eclampsia*. Minerva Ginecologica 4 553 Nov. 1952.

780. MAURIZIO E. *Vitamin B₁₂ (cyanocobalamin)* in the medical therapy of toxemia of pregnancy and eclampsia, *Minerva med.* 1 571-572, March 7 1953.

The author states that in patients with toxemia of pregnancy and eclampsia medical management is designed to combat the three derangements always present in each variety of the eclamptic syndrome, namely convulsions, angiospasm and toxicity. He points out that as a result of widespread alterations in the general metabolism of the body the toxic state involves the entire organism. The mechanism of detoxification is accomplished by means of a large mass of oxidative, reductive and conjugative reactions which take place principally in the liver and kidneys. Detoxification therefore rests, to a large extent, upon the functional integrity of these organs.

The author's "detoxification" regimen in these patients is discussed in considerable detail. Mention is made of his experiences, dating from 1946, with the use of nicotinic acid and liver extracts in the treatment of the "toxicity state" of these patients. The author avers that his results with these agents over a period of four years were good. He explains that the actions expected from the use of nicotinic acid were two: (a) a hypotensive effect; (b) a "hepato-protective" effect. The presupposition upon which his use of nicotinic acid was made in these patients is based upon the thought that this chemical, which has coenzyme activity, participates in the formation of an important enzymatic system which intervenes in respiratory-cellular processes, that is coenzyme I and

H, which activate redox processes of the liver cell. (In general, the doses of nicotinic acid employed varied from 50 to 150 mg. daily; rarely were doses of 200 mg. daily given.)

Impressed by the clinical efficacy of liver extracts in the eclamptic syndrome, the author began to use vitamin B₁₂ in an effort to obtain hepatoprotective and detoxification effects in these patients. Recent researches, evaluated by the author in which B₁₂ was employed in the treatment of patients with the eclamptic syndrome, revealed that the vitamin has biologic activity which the author summarizes as follows: 1. Stimulates the synthesis of nucleic acids. 2. Favors transmethylation. 3. Develops lipotropic activity. In an attempt to elucidate the mechanism of the action of B₁₂, the author found it to possess a pronounced "hepatotropic" and "nephrotropic" effect, stimulating a remarkable increase in the activity of "beta-glucuronidase" and an "aspecific cholinesterase" of the liver and kidneys.

Based upon these observations the author treated "numerous patients" with "toxicity of pregnancy" with doses of B₁₂ which varied from 20 to 50 mcg. daily more frequently doses over 30 mcg. were used. "Consistently satisfactory" results were obtained as evidenced by a notable improvement in the patient's general condition and in liver and kidney function. The efficacy of this therapy was observed within 24 to 48 hours. In none of the patients treated were eclamptic convulsions seen. "An increased diuresis was observed in all patients treated."

The University of Genoa
Genoa, Italy

ANIMAL STUDIES

PHYSIOLOGIC AGENTS

781. TRAINA, V. *Vitamin B₁₂ and Histamine*, *Nature* 165 439-440, March 18, 1950 (in Letters to the Editors)

Four guinea pigs, serving as controls, were given intracardiac injection of 0.12 mg. of histamine (almost twice the LD₅₀ by intravenous injection). Within half an hour all of them had died of shock. Eight more guinea pigs were given 15 mcg. of vitamin B₁₂ intraperitoneally; later each received a 1-mg. intracardiac injection of histamine. None of them died, although very slight signs of shock were noted. It is the writer's impression that the same dose of B₁₂ was more effective in histamine shock than in anaphylactic shock in the guinea pig. From a study of the contraction response of guinea pig intestine *in vitro*, the writer concluded that vitamin B₁₂ does not act by direct inactivation of histamine. In another experiment on guinea pigs the writer obtained results which indicate that the B₁₂ does not activate histaminase. On the basis of the experiments cited, it is concluded that the mechanism of action of B₁₂ seems to be competitive antagonism as in the case of the common antihistaminic drugs.

ROME, I T

782. TRAINA, V. *Vitamin B₁₂ as an anti-anaphylactic*, *Nature* 166 78-79 July 8 1950.

Following the hypothesis that macrocytic anemia could be due to an anaphylactic blockage of the bone marrow experiments were conducted which showed that vitamin B₁₂ had a protective action against anaphylactic shock in sensitized guinea pigs when it was administered 15 minutes prior to a shocking dose of horse serum. It was effective in doses as low as 60 to 90 mcg. per Kg. of body weight. This dose is one-thirtieth to one-fiftieth as large as that required when antihistaminic agents are used to protect guinea pigs from anaphylactic shock.

Feltrina Park Hospital
Cleveland, Ohio

783. SHARPE, H. M., WOOLLETT, E. A., and CUTHBERTSON, W. F. J. *Vitamin B₁₂ and antihistaminic activity*, *Nature* 166 651-652, Oct. 14 1950 (Letter to Editors)

Subcutaneous injection of 50 to 500 mcg. of vitamin B₁₂ did not protect guinea pigs from having a fall in blood pressure after intravenous doses of histamine. Subcutaneous injection of 15 mcg. of vitamin B₁₂ per Kg. of body weight did not protect these animals from bronchospasm, often fatal, when histamine was injected intracardially.

Chen Laboratories Ltd.
Greenford, Middlesex, England

784. DUCROT, R. *Vitamin B₁₂ and histamine*, *Compt. rend. Soc. de biol.* 144 697 1950 (*Allergy Abstr.* 16 94, in *J. Allergy* 22 Nov 1951)

"Vitamin B₁₂, which is said to have an antihistaminic action, failed to exert any protective effect against histamine shock induced in guinea pigs by the inhalation of aerosolized histamine or by the intravenous injection of histamine. Vitamin B₁₂ also failed to show antihistaminic properties in Dale experiments with isolated guinea pig intestine."

785. NARANJO P: *Histamine shock and vitamins* Proc. Soc. Exper. Biol. & Med. 81 111 113, Oct. 1952.

Author's summary: "1 Even in high doses, vit. B₁, B₁₂, C, K, and B complex did not protect guinea pigs against histamine shock produced by inhalatory administration of histamine aerosol. 2 Vit. B₁₂ and C did not exhibit any antihistaminic action in assays of blood pressure in cats and guinea pigs. 3 Vit. B₁₂ and C did not show any antihistaminic action in assays on the isolated guinea pig ileum. In high doses both vitamins caused the intestinal strip to relax, but without decrease of its histamine sensitivity."

Central University of Ecuador
Quito, Ecuador

786. LUDOVICI, P. P., and AXELROD A. E. *Circulating antibodies in vitamin-deficiency states. Pteroylglytamic acid, niacin-tryptophan, vitamins B₁₂, A and D deficiencies*, Proc. Soc. Exper. Biol. & Med. 77 526-530, July 1951

Authors summary: "1 Hemagglutinin production in response to inoculation with human erythrocytes has been investigated in pteroylglytamic acid, niacin-tryptophan, vit. B₁₂, vit. A, vit. D-deficient rats and their respective controls. 2 Severe impairment of antibody response was observed in the pteroylglytamic acid-deficient rats. Moderate impairment was demonstrated in vit. A and niacin-tryptophan deficiencies. Vit. B₁₂ and vit. D deficiencies had no effect on antibody formation. 3 A rough classification of the effect of 10 deficiency states upon antibody production is presented."

University of Pittsburgh
Pittsburgh, Pa.

787. AXELROD A. E.: *Role of the vitamins in antibody production (A examination of the author's data to date)* Metabolism 2 1-8, Jan. 1953.

Author's summary: "The available evidence would indicate that certain B vitamins, notably pyridoxine, pantothenic acid and pteroylglytamic acid, play a significant role in antibody synthesis. There is considerable basis for linking pyridoxine to amino acid metabolism, and its participation in antibody production may be related to this function. A corresponding mechanism for the action of pantothenic acid has not yet been demonstrated. A relationship between pantothenic acid and peptide bond formation is suggested by the observation of Chantreune that coenzyme A, a compound that contains pantothenic acid, is involved in the synthesis of hippuric acid."

"The ability to control antibody production by regulating the vitamin intake should be a useful tool in the unraveling of the mechanisms of antibody synthesis. Since the antibodies represent a class of specialized proteins, such studies would yield information regarding the

processes of protein anabolism in general. The fact that the measurement of antibodies is greatly facilitated by the application of specific immunological procedures renders them particularly suitable for studies of protein synthesis."

In the experiments referred to, in which mice were used, neither vitamin B₁₂ nor vitamin D deficiency states had any effect on antibody formation. There was moderate impairment of antibody formation in mice which were deficient in riboflavin, thiamine, biotin, vitamin A and niacin-tryptophane.

Institute of Pathology and
Western Reserve University
Cleveland, Ohio

788. OGASAWARA, K., ATA, S., HISADA, S. *Vitamin B₁₂ and acetylcholine shock* Lancet 1 598-599 March 21 1953 (in Letters to the Editor)

In experiments with mice weighing 12 Gm., it was found that 15 mcg. of vitamin B₁₂ given intraperitoneally 15 to 90 minutes before an MLD (minimum lethal dose) of histamine given intravenously was sufficient to protect all mice from death and from most signs of shock. When the vitamin was given 120 minutes before the histamine there was no protection from shock and death.

Mice kept for a week on a deficient diet showed less resistance to histamine (MLD was halved) but 15 mcg. of vitamin B₁₂ intraperitoneally protected them from 1.7 MLD of histamine given 15 minutes later.

In the doses given (15 and 30 mcg.) vitamin B₁₂ intraperitoneally did not protect the mice from shock and death from acetylcholine (15 mg. per kg. or MLD). However when 12 mcg. of vitamin B₁₂ was given intravenously at the same time as the acetylcholine, all mice survived. All died when the amount of vitamin was decreased or acetylcholine increased.

In rabbits it was found that 5 to 30 mcg. of vitamin B₁₂ per Kg. given 5 minutes before 5 mg. of acetylcholine per Kg. did not modify the effect of acetylcholine on blood pressure.

Yagyu University School of Medicine
Japan

789. MEYER, I. K., and DAVIS, J. E. *Anemia produced by acetylcholine in guinea pigs*, Federation Proc. 12 350 March 1953.

"Eight normal male guinea pigs which had received a uniform diet of Purina Rabbit Chow were found to have red blood cell counts of approximately 6 million. After determining the correct resting erythrocyte number for each animal, we administered daily subcutaneous injections of acetylcholine bromide (2 mg.) This caused reductions in the erythrocyte numbers of all of the animals. Normal counts were diminished by 29-71% (average 49.6) within 21-67 days. These decreases represent the maximum degrees of anemia which were reached in an average period of 35 days of acetylcholine injections. Three of the animals died at times during which their anemias were most severe. One animal showed a peak reticulocytosis of 21% on the 4th day of therapy with vitamin B₁₂ in spite of daily injections of acetylcholine. However neither vitamin B₁₂ nor folic acid alone

found to cause complete regeneration of the red blood cells. In a few animals combined treatment with both folic acid and vitamin B₁₂ resulted in the restoration of the erythrocyte numbers almost to normal, in spite of continued daily injections of acetylcholine. The cellular type of this experimental anemia was not studied, since our blood samples were obtained by puncturing the small veins of the ear.¹⁰

University of Texas College of Pharmacy
Austin, Texas

Drug Toxicity

- 790 BOSSHARDT D. K., HUFF J. W., PAUL, W. J., and BARNES B. H.: *Animal protein factor and growth of mice fed diets containing succinylsulphisalazole*, Federation Proc. 8 378, March 1949

Sherry & Doherty, Inc.
Cinn. Ohio, Pa.

- 791 JONES, J. H., ROGERS, C. S., and STONE, C. H.: *Succinylsulphisalazole and a rat growth factor in liver* J. Nutrition 39 579-590, Dec. 1949

Young rats grow well on a synthetic diet containing purified casein and all members of the B complex except vitamin B₁₂ and folic acid. The addition of succinylsulphisalazole caused failure of growth, leukocytopenia, and then death. The inclusion of folic acid in the succinylsulphisalazole diet corrected the blood dyscrasia and prevented the death of the animals but failed to restore growth completely. Animals given succinylsulphisalazole plus a crude liver extract had normal blood and grew rapidly. The subcutaneous injection of a purified antipernicious anemia liver preparation into rats on the diet containing succinylsulphisalazole and folic acid produced very good growth. The authors are unable to conclude definitely that vitamin B₁₂ is the factor in liver responsible for the increased growth in the rats receiving succinylsulphisalazole, but believe that this is the case because of the fact that only 0.02 cc. of an antipernicious anemia concentrate produced excellent growth in the rats receiving succinylsulphisalazole and folic acid.

University of Pennsylvania School of Medicine
Philadelphia, Pa.

792. ASENJO C. F.: *Influence of vitamin B₁₂ on a succinylsulphisalazole-induced dietary deficiency in rats* Federation Proc. 9 147-148, March 1950.

"In the course of investigating the supplementary action of natural products on folic acid-deficient rats receiving a highly purified basal diet containing succinylsulphisalazole, it was noticed that many of the products tested induced a growth response and a leukocyte regeneration at rates well above those obtained with the optimal level of folic acid. This suggested that the depleted animals suffered not only from a severe folic acid deficiency but also, perhaps, from other less severe deficiency or deficiencies. As such a basal diet could have possibly developed a vitamin B₁₂ deficiency concurrently with the severe folic acid deficiency it was decided to study the effect that B₁₂ supplementation could have in the depleted rats. Preliminary evidence indicates that when high levels of folic acid are given as supplement, the concurrent administration of vitamin B₁₂ significantly improved the growth-response and white cell count. At low levels of

folic acid supplementation, the addition of B₁₂ did not improve the response produced by folic acid alone, though survival was a little longer. Folic acid negative controls receiving B₁₂ did not show any improvement, but they also lasted longer than the controls without B₁₂. The incidence of infarct like splenic lesions was high in both groups of controls, in those receiving B₁₂, perhaps because they survived longer the splenic lesions developed to such an extent that this organ was found, in several instances, adhered to those adjacent. A level of 0.5 µg. [per] day of B₁₂ was used throughout."

School of Tropical Medicine
University of Puerto Rico
San Juan, P. R.

793. FÖLLMER, W. *Sulfonamides and fetal development*, Mod. Welt 20 53- Jan. 13, 1951 (abstr. J.A.M.A. 145 1900, April 21, 1951)

"According to Föllmer sulfonamides administered to a pregnant animal pass the placental barrier and reach the fetus. When the administration is prolonged, the concentration in the fetal serum is about the same as that in the maternal serum. Experiments are described that indicate that prolonged administration of sulfonamides during pregnancy exerts a damaging effect on the fetus, but this harmful effect becomes manifest chiefly after delivery in that growth in length and increase in weight are retarded in the young animals. Apparently compensatory mechanisms in the maternal organism prevent serious damage to the fetus during the intrauterine life, because experiments on tadpoles demonstrated that sulfonamides do inhibit the growth of embryonic tissues. Thus the sulfonamides not only exert bacteriostatic effects but also cytostatic effects on embryonic tissues. The tadpole test, which the author describes, apparently is capable of demonstrating cytostatic effects of various substances, such as vitamin B₁₂ and choline, on embryonic tissues."

- 794 ERSHOFF B. H.: *Growth-promoting activity of vitamin B₁₂ in mice fed massive doses of streptin* Arch. Biochem. 26 50-53, March 1950.

Growth retardation in mice due to massive doses of streptin was only partially counteracted by vitamin B₁₂ while extracted liver residues restored growth to normal. This indicates the presence of a factor(s) in liver other than vitamin B₁₂ which contributes to growth promotion. It is distinct from any known B vitamin.

795. ATA, S., and TANAKA, K.: *Vitamin B₁₂ and isoniazid*, Lancet 2 589 Sept. 20, 1952 (in Letters to the Editor)

Experiments with mice are described in which it was shown that large doses of vitamin B₁₂ decreased the toxicity of isoniazid. Most effective were doses of 800 mcg./Kg. body weight whether given half an hour before, half an hour after or with a subcutaneous dose of isoniazid. Similar experiments using 30 and 3 mcg. of vitamin B₁₂ per Kg. of body weight administered half an hour after the isoniazid had only a slight effect on the LD₅₀ of the isoniazid in mice.

Nagoya University School of Medicine
Japan

796. RUBIN B., and BURKE, J. C.: *Vitamin B₁₂ and Isoniazid* Lancet 2 937 Nov 8, 1952 (in Letters to the Editor)

"Recently Aita and Tanaka reported that a subcutaneous injection of vitamin B₁₂ (0.30 mg per kg. body weight) appreciably diminished the acute toxicity of isoniazid in mice. In their experiments a definite increase in the subcutaneous L.D.₅₀ dose of isoniazid was obtained when the above-mentioned dose of B₁₂ was administered thirty minutes after the isoniazid injection. We have been unable to confirm their results using aqueous solutions of crystalline vitamin B₁₂. Even a 16-fold increase in the dose of B₁₂ failed to protect against the acute toxic effects of isoniazid in male albino mice. Isoniazid was administered as a 2% aqueous solution and vitamin B₁₂ was injected as a 0.002 or 0.032% aqueous solution. The B₁₂ test solutions were checked for qualitative and quantitative content by infra red and ultraviolet absorption measurements. Dose volumes of the B₁₂ solutions injected were 0.30 ml. per 20 g. body weight. Isoniazid control mice received equivalent volumes of physiological saline subcutaneously instead of B₁₂ solution. The results are shown in the following table:

EFFECT OF VITAMIN B₁₂ ON TOXICITY OF ISONIAZID IN ALBINO MICE

Subcutaneous 20 minutes after treatment		Subcutaneous Isoniazid L.D. ₅₀ (mg. per kg.)	No. of Mice
Dose	(mg. per kg.)		
Saline	—	790 (779-813)	30
B ₁₂	0.30	126 (100-151)	30
B ₁₂	4.80	125 (767-866)	30

25/30 mice showed lesions indicated in parentheses.

Neither of the two B₁₂-isoniazid L.D.₅₀ doses differed significantly from the saline-isoniazid L.D.₅₀.

"Among more than fifty compounds screened as possible prophylactics against isoniazid acute toxicity in mice, only barbiturates and some related central depressant agents appeared to offer therapeutically useful protection. Certain thiol compounds, including dimercaprol (BAL) offered only a transient suppressive action in high doses. A large series of other agents, which included carbohydrate metabolites, amino-acids, and vitamins, failed to protect mice unequivocally against the acute toxic effects of isoniazid. These results are to be published in detail elsewhere at a later date."

Smith Institute for Medical Research
New Brunswick, N. J.

PRODUCTS OF BODY METABOLISM

797. MENGE, H., and COMBS, G. F.: *Action of vitamin B₁₂ in counteracting glycine toxicity in the chick*, Proc. Soc. Exper. Biol. & Med. 75 139-142, Oct. 1950.

The results indicate that vitamin B₁₂ functions in the metabolism of glycine.

798. STERN, J. R., and MCGINNIS, J.: *Toxicity of glycine for vitamin B₁₂-deficient chicks*, Proc. Soc. Exper. Biol. & Med. 76: 233-234, Feb. 1951.

Previous reports have indicated that vitamin B₁₂ is concerned with nitrogen utilization and metabolism. This

was investigated by feeding chicks which had received vitamin B₁₂ and vitamin B₁₂-deficient chicks a known amount of nonprotein nitrogen (1 Gm. of glycine in a gelatin capsule, force fed). Both plasma nonprotein nitrogen and amino nitrogen levels determined at two and four hours after administration of glycine showed that the birds which had received vitamin B₁₂ were better able to metabolize the glycine than were the B₁₂-deficient birds. There were evidences of toxicity of glycine in the B₁₂-deficient birds. When food was withdrawn from the chicks the night before the glycine was administered, the glycine was much more toxic to the B₁₂-deficient birds than when they were allowed food, those which had received B₁₂ were able to withstand glycine with or without food.

Vanderbilt State College
Palmers, Wash.

799. MACHLIN, L. J., DENTON, C. A., and BIRD, H. R.: *Effect of vitamin B₁₂ and folic acid on glycine toxicity in the chick*, Federation Proc. 10 553, March 1951.

"Day-old chicks from dams fed a vitamin B₁₂ low ration were placed on diets consisting primarily of corn and soybean meal for 4 to 6 weeks. Glycine was added at levels of 8, 6, and 9% with and without the addition of crystalline vitamin B₁₂. The chicks were able to tolerate up to 6% glycine in the presence of B₁₂, whereas 5% was definitely toxic in its absence. In the second experiment, glycine was added at levels of 6 and 9% and the B₁₂ content of the diets was varied. The apparent requirement for B₁₂ was increased by the addition of glycine. In the third experiment, a 6% level of glycine was used and B₁₂, folic acid, choline, or a combination of folic acid and choline were added. Folic acid, in the presence of choline, alleviated the glycine toxicity. In the 4th experiment, the diets contained B₁₂ and folic acid, alone and in combination, with and without the addition of 6% of glycine. Folic acid again helped to counteract the toxicity, although not as much as did the B₁₂. When both were added, the chicks grew almost as well as those receiving no glycine. The uric acid content of the blood was raised either by glycine or B₁₂. It was raised more by the two in combination than by either alone. Folic acid reversed this effect and decreased blood uric acid."

U. S. Department of Agriculture
Beltsville, Md.

800. HARDIN, J. O., and ROVE, E. L.: *Prevention of DL-methionine toxicity in rats by vitamins E, B₁₂, folic acid, glycine and arginine*, Proc. Soc. Exper. Biol. & Med. 78 728-731, Dec. 1951.

Authors summary: "(1) The toxicity of DL-methionine fed to rats at a 2% dietary excess level, was more pronounced in the absence of dietary vit. E, folic acid, and vit. B₁₂. The combination of vit. E and folic acid was as effective for growth as all 3 vitamins together. Without the 3 vitamins, growth in 4 weeks was 32% of the normal, as compared with 54% of normal upon their addition to the diet. Supplements of glycine and arginine in the absence of these vitamins produced growth that was 62% of normal. (2) The methionine toxicity was prevented by molar equivalent supplement levels of glycine plus arginine if the 3 vitamins were added to the diet (concomitantly)."

normal) but was not prevented in the absence of these factors. (3) Urinary creatinine and sensitivity to acute carbon tetrachloride were determined for all rats. (4) complete protection against glycine toxicity was given by dietary methionine with vit. E."

*Johnson Polytechnic Institute
Asheville, N.C.*

801. SURE, R., and EASTERLING L. *The protective action of vitamin B₁₂ against the toxicity of DL thyroxine*, J Nutrition 42 221-225, Oct. 1950

Studies in rats revealed that as little as one part of crystalline vitamin B₁₂ affords 100 per cent protection against a toxic fatal dose of 666 parts of thyroxine. I.e., 0.3 mcg. of vitamin B₁₂ protects against 200 mcg. of thyroxine.

*University of Arkansas
Fayetteville, Ark.*

802. ERSHOFF B. H. *Beneficial effects of liver on cortisone acetate toxicity in the rat*, Proc. Soc. Exper Biol. & Med. 78 836-840, Dec. 1951

The toxic effects of large doses of cortisone acetate were largely counteracted in the rat by the administration of desiccated whole liver. Liver did not counteract the effect of the drug on the leukocyte count. The beneficial effect of liver was apparently not due to its content of known B vitamins.

Supplements of all known B vitamins (B₁₂ at level of 150/Kg. of ration) were without significant effect on the gain in body weight of immature rats fed 100 mg/Kg. of diet of cortisone acetate.

Other levels of cortisone used 200 and 400 mg/Kg. of diet. Females were more severely affected by cortisone than males. Females benefited some by liver.

*Lowry F. Thomson Laboratories
Los Angeles, Calif.*

ORGANIC, INORGANIC CHEMICALS

803. POPPER, H., KOCH WESER, D., and SZANTO P. B. *Protective effect of vitamin B₁₂ upon hepatic injury produced by carbon tetrachloride*, Proc. Soc. Exper Biol. & Med. 71 688-690, Aug. 1949

The administration of vitamin B₁₂ (15 mcg. per 100 Gm. of body weight) to rats before acute carbon tetrachloride intoxication inhibits the development of histologic changes, especially fatty metamorphosis and depletion of ribonucleic acid. In addition, the deposition of lipids (determined biochemically) and the bromsulphalein retention is less than in the intoxicated controls. These results may be related to an effect of vitamin B₁₂ on cytoplasmic ribonucleic acid, which has been shown to disappear early in hepatic injury.

*National Institute for Medical Research,
Cecil County Hospital, and
Yale University University Medical School
Chicago, Ill.*

804. KOCH WESER, D., and POPPER, H.: *Correlation between ribose nucleic acid depletion and other signs of liver damage as influenced by vitamin B₁₂*, Proc. Central Soc. Clin. Research 22 91-92, Nov. 1949 J Lab. & Clin. Med. 34 1764-1765, Dec. 1949 (In Soc. Proc.)

Since disappearance of cytoplasmic ribonucleic acid compounds, which supposedly are important to protein formation, is one of the first signs of liver damage, the authors studied ribonucleic acid depletion in 86 rats previously injected intraperitoneally with carbon tetrachloride. The histologic signs of ribonucleic acid depletion were then correlated with other signs of liver damage. Recent investigations had suggested that vitamin B₁₂ promotes the formation of ribonucleic acid compounds and it had been shown that the effects of carbon tetrachloride poisoning were modified in rats that had previously received large doses of this vitamin. Therefore, 48 rats thus protected were included in this study. Since small doses of carbon tetrachloride produced ribonucleic acid depletion in the center of the lobules without other evidence of liver damage, it appears that ribonucleic acid depletion is one of the basic factors in this type of liver damage. Moreover vitamin B₁₂ seems to inhibit liver damage by facilitating the formation of ribonucleic acid. This action is probably related to the supposed role of growth factors such as vitamin B₁₂ as synthesizing ribonucleases. Their influence on protein formation may in turn control the enzyme regeneration in liver damage.

In view of these findings, it is felt that the effect of vitamin B₁₂ in different types of liver diseases should be investigated. Considering the large doses of this vitamin required to protect rats against liver damage, the variations in B₁₂ content of liver extract may explain the erratic results the extract produces in the treatment of liver disorders.

805. KOCH WESER, D., SZANTO P. B., FARBER, E., and POPPER, H.: *Further investigation on the effect of vitamin B₁₂ concentrate upon hepatic injury produced by carbon tetrachloride*, J Lab. & Clin Med. 36 694-704 Nov 1950.

Bromsulphalein retention, increase in liver weight and increase in total lipids due to CCl₄ was lessened by the administration of 15 mcg. B₁₂/100 Gm. body weight—given in 4 divided doses before administration of CCl₄, small amounts before or large doses after CCl₄ were ineffective.

Histologically there appeared to be less fatty metamorphosis and central necrosis and less loss of pyroninophilia in the rats given vitamin B₁₂.

Prior administration of vitamin B₁₂ concentrate increased the lethal dose of CCl₄ significantly. Action of B₁₂ as a pharmacologic vasodilator was considered.

806. MUSHETT C. W. *Influence of crystalline vitamin B₁₂ on carbon tetrachloride poisoning* Federation Proc. 9 339 March 1950.

"In confirmation of results obtained by Popper and associates (Proc. Soc. Exper Biol. & Med. 71: 688, 1949 [Abstr. 903]) with vitamin B₁₂ concentrate, it has been found that crystalline vitamin B₁₂ exerts a protective effect against the fatty metamorphosis, hydrophobic change and depletion of cytoplasmic ribonucleic acid in the livers of rats given a single oral dose of carbon tetrachloride. However when both carbon tetrachloride and vitamin B₁₂ were given daily for a period of 3 weeks vitamin B₁₂ failed to prevent the hepatic changes even at a dose level

of 200 µg. In fact, the livers of the B₁₂ treated rats were relatively larger and contained more lipid than those of rats given carbon tetrachloride alone. Despite its inability to protect the livers of the poisoned rats, vitamin B₁₂ even at low doses, enabled the treated animals to grow at a considerably greater rate than the rats not treated with the vitamin. An explanation for the growth effect was found not to be due to differences in renal damage since this was negligible in both groups. In dogs given repeated doses of carbon tetrachloride vitamin B₁₂ failed to show a protective effect during life as judged by morphologic blood picture, prothrombin time, icteric index and blood biochemical determinations. The continuation of vitamin B₁₂ treatment, however after cessation of carbon tetrachloride dosing apparently brought about a more rapid reversibility of hepatic changes as indicated by gross and microscopic examination of the livers of the dogs."

*British Institute for Therapeutic Research
Belmont, N. Y.*

- 807 HOVE, E. L., and HARDIN J. O. *Creatine formation in CCl₄ poisoned rats as influenced by dietary α-tocopherol, vitamin B₁₂, and cystine* Federation Proc. 10 385, March 1951.

"Rats reared on synthetic type diets with 10% casein were subjected to weekly sub-lethal CCl₄ injections. Supplements of L-cystine, DL-α-tocopherol acetate and vitamin B₁₂ in all combinations were added, or the casein was increased to 18% with and without tocopherol. The creatinuria associated with the vitamin E deficiency was doubled by the CCl₄ injections among rats on the 10% casein diet but was not influenced in rats on the 18% casein diet, or in rats on the 10% basal diet supplemented with cystine. Vitamin B₁₂ increased the creatinuria except in the presence of both cystine and vitamin E. Determinations were made on the ability of liver slices from rats on the various dietaries to synthesize creatine, *in vitro* from guanidoacetic acid and methionine. Conditions resulting in high creatinuria such as vitamin E deficiency or CCl₄ toxicity were associated with low creatine synthesis by liver slices, except when vitamin B₁₂ was included in the diet. In these cases not only was the synthetic activity of the liver increased, but methionine was not necessary in the substrates. The conclusion drawn from the results on growth, growth deficit due to CCl₄, creatinuria, tooth color and other criteria is that the 10% casein diet is made equal to the 18% casein diet by the addition of both cystine and vitamin B₁₂. In the absence of these factors the vitamin E requirement of the rat is increased and vitamin E is involved in the synthesis of creatine from guanidoacetic acid and labile methyl."

*Dalhousie Polytechnic Institute
Halifax, N.S.*

808. HOVE, E. L., and HARDIN J. O. *Effect of vitamins E, B₁₂, and folic acid on CCl₄ toxicity and protein utilization in rats* Proc. Soc. Exper. Biol. & Med. 77: 502-505, July 1951.

Authors summary "Vit. E and vit. B₁₂ can replace each other in promoting protein utilization and in protecting against acute carbon tetrachloride toxicity in young rats reared on 10% protein diets deficient in these factors. The vit. B₁₂ requirement for protection against CCl₄ is considerably higher than for growth. Folic acid

supplements, alone, had no effect, but, in the presence of vit. B₁₂ or vit. E, this factor markedly improved protein utilization and growth. L-Cystine protected against the acute toxicity but a synergism between cystine and vit. E was not noted."

*Dalhousie Polytechnic Institute
Halifax, N.S.*

- 809 HOVE, E. L., and HARDIN J. O. *Effect of vitamin E and CCl₄ on fat, respiration and choline oxidase of rat livers*, Proc. Soc. Exper. Biol. & Med. 78 858-861 Dec. 1951.

Both vitamin E and vitamin B₁₂ have been found to give protection against liver damage induced in rats by carbon tetrachloride. Since carbon tetrachloride is known to produce an immediate fatty infiltration of the liver and an increased rate of respiration of liver slices from guinea pigs poisoned with carbon tetrachloride has been reported by Ennor (*Australian J. Exper. Biol. & M. Sc.* 20 78 1942) the influence of vitamin E and vitamin B₁₂ on the response of liver fat and the rate of liver slice respiration after acute carbon tetrachloride poisoning was investigated in rats. The liver fat of rats on a 10 per cent casein diet was significantly lower in animals that received no vitamin E than in those that were given this vitamin, but no difference was noted in the treated and untreated animals when the diet contained 18 per cent casein. Following injections of carbon tetrachloride the liver fat increased sharply to about the same degree on all diets. Although the fatty changes 24 hours after the injections were not affected by the vitamin supplements, these supplements, especially vitamin B₁₂, permitted a more rapid return to normal liver fat values. Increase of respiration of liver slices from animals injected with carbon tetrachloride was not substantially inhibited by dietary supplements of vitamin E. The choline oxidase activity of the liver was not influenced by dietary vitamin E or by carbon chloride injections into rats, but increased dietary fat level increased the activity of this enzyme system.

*Dalhousie Polytechnic Institute
Halifax, N.S.*

810. ERSHOFF, B. H. *Comparative effects of B vitamins and liver on dinitrophenol toxicity in the rat*, J. Nutrition 42 271-277 Oct. 1950.

When 0.1 per cent dinitrophenol was added to the diet of rats, retardation in body and testicular weights was produced. These effects were completely counteracted by the administration of desiccated whole liver. Supplements of all of the known B vitamins, including vitamin B₁₂ had little, if any effect. It is pointed out, however that the vitamin B₁₂ content of the whole liver ration was considerably greater than the B₁₂ content of the diet containing the synthetic vitamin supplements. It is possible that the protective effects of the whole liver ration may have been partly due to its higher vitamin B₁₂ content.

*University of Southern California
Los Angeles, Calif.*

811. FISHER, B., and VARS, H. M. *Effect of vitamin B₁₂ and aureomycin upon the toxicity of chloroform to regenerating liver* Federation Proc. 10 354, March 1951.

"Adult male Wistar rats were fed a non-protein diet for 14 days, subjected to 70% partial hepatectomy and offered an 18% fibrin diet. Group A received 10 mg. aureomycin per gram of food eaten. Group B was injected with 2 γ vitamin B₁₂ on alternate days (total dose 8 γ) and group C received saline injections. On the 4th postoperative day all received a subcutaneous injection of chloroform (1.2 ml/kg.) in mineral oil (1 ml. volume) and were sacrificed 4 days later. All groups ate approximately the same amount of food. There was no significant difference between the groups in the amount of liver protein regeneration or in the histological picture as observed by hematoxylin and eosin, or methyl-green and pyronin stains. Varying the time intervals of therapy and sacrifice did not change the results. We conclude that under these conditions neither vitamin B₁₂ nor aureomycin exerted either a protective action to the liver or a stimulus to liver protein regeneration."

University of Pennsylvania School of Medicine
Philadelphia, Pa.

812. DiPORTO A., and SEBASTIANO, M. *Action of vitamin B₁₂ supplemented by vitamin C and vitamin K in experimental intoxication due to benzene hematological aspects*, Policlinico 58 172 June 1951

813. STOKINGER, H. E., and STROUD M. T.: *Anemia in acute experimental beryllium poisoning*, J Lab & Clin. Med. 48 173-182, Aug. 1951.

A mild anemia was produced in dogs, rats, and rabbits by exposing them to an aqueous aerosol of beryllium fluoride. The anemia resembled a macrocytic anemia and differed according to the species. In dogs the red blood cell count, mean corpuscular volume, and hemoglobin all changed as in typical normochromic macrocytic anemia. In rabbits there was a lessened tendency for the hemoglobin concentration of the whole blood to decrease and the tendency to return to normal was more pronounced. In rats the hemoglobin values were normal while the other two values changed as in macrocytic anemia.

Vitamin B₁₂ was given to 2 dogs during the period of exposure to the beryllium mist, and to 2 others after 28 weeks of exposures to the mist none of the dogs received any benefit from the vitamin. Another group of dogs was given liver-stomach concentrate for a month before, and during the period of, exposures to the beryllium salt aerosol. These dogs fared worse than did the control dogs. Folic acid given at the time the anemia was being induced was successful in treating rats but appeared to contribute to the death of one of the dogs that received it.

In the discussion the authors state "A possible explanation for the detrimental effect of [the liver-stomach concentrate] on the anemia in dogs might be that its iron, a known gastric irritant, further added to the irritant effect of beryllium brought to the gastrointestinal tract by ciliary action in the respiratory system. Whether a similar explanation holds for folic acid is not known."

University of Rochester
Rochester, N. Y.

814. MUSHETT C. W., KELLEY K. L., BOXER, G. E., and RICKARDS J. C.: *Antidotal efficacy of vitamin B₁₂ (hydroxo-cobalamin) in experimental cyanide poisoning*, Proc. Soc. Exper. Biol. & Med. 81 234-237 Oct. 1952.

Authors summary and conclusions: "1. Vit. B₁₂ (hydroxo-cobalamin) but not vitamin B₁₂ (cyano-cobalamin), has been found to be capable of preventing in mice the toxic symptoms and death due to cyanide administration. 2. When injected into mice exhibiting complete respiratory arrest and coma due to cyanide poisoning, vit. B₁₂ effected rapid recovery of most animals. 3. In mice injected with potassium cyanide followed by vit. B₁₂, some of the cyanide appears in the urine as thiocyanate, but a greater percentage of the cyanide appears as vit. B₁₂, having formed this compound by reacting with the vit. B₁₂."

Marsh Institute for Therapeutic Research, and
Marsh & Co., Inc.
Rahway, N. J.

815. ANDREWS, G. C., POST, C. F., and DOMONKOS, A. N. *Seborrheic dermatitis: supplemental treatment with Vitamin B₁₂*, New York State. J. Med. 50 1921-1925, Aug. 15, 1950

The authors consider seborrheic dermatitis to be essentially a nutritional disease, due to an underlying digestive deficiency. This can be corrected by vitamin B complex and crude liver extract therapy. Folic acid has been used, but does not give as good results as crude liver extract. Many patients with seborrheic dermatitis are infected by *Pityrosporon* or *staphylococci*. It may be assumed that the resistance of these patients to infection has been lowered by nutritional or metabolic disturbances, which can be overcome by treatment with crude liver extract, vitamin B complex, folic acid, or vitamin B₁₂.

In the past year the authors have used vitamin B₁₂ and vitamin B₁₂ concentrate parenterally in the treatment of 101 dermatologic patients. The dosage was 10 to 30 mcg. once a week or in some cases, every two to three weeks. Secondary infections of the seborrheic areas were treated with the sulfonamides or penicillin. Local treatment was given as necessary. Of the 101 patients, 66 were followed sufficiently for evaluation of the effects of vitamin B₁₂. In 16 of the 37 cases of seborrheic dermatitis, and in each of the single cases of chronic radiodermatitis and psoriasisform dermatitis there was great improvement, usually after two or three injections. Many of these patients have remained entirely free of seborrheic dermatitis. Mild recurrences were usually satisfactorily treated with a few additional vitamin B₁₂ injections, and recurrences have been prevented entirely by a maintenance dose of 15 or 30 mcg. every two or three weeks.

*Presbyterian Hospital, and
Columbia University College of Physicians and Surgeons
New York, N. Y.*

816. ANDREWS, G. C., POST, C. F., and DOMONKOS, A. N. *Advances in the treatment of seborrheic dermatitis and acne vulgaris*, Australian J. Dermat. 1 11 March 1951 (abstr. Am. Pract. 3 256, March 1952)

Vitamin B₁₂ administered intramuscularly in doses of 10 to 30 mcg. once a week or in some cases, once every two to three weeks, as an adjunct to local treatment, has brought about improvement in a large number of patients with seborrheic dermatitis after two or three injections. Many patients have had no recurrences. A mild recurrence can usually be treated satisfactorily with a few additional injections, and recurrences have been prevented entirely by a maintenance dose of 15 or 30 mcg. every two or three weeks. The concentrate and the crystalline preparation had similar action. Larger doses are necessary to bring about a response in patients with the skin disease than in those with pernicious anemia.

New York, N. Y.

817. ANDREWS, G. C., DOMONKOS, A. N., and POST, C. F. *Treatment of acne vulgaris*, J.A.M.A. 146 1107-1113, July 21 1951.

For two years the authors have treated acne vulgaris by means of antibiotics and estrogens and have not employed X ray therapy. Results are given for 384 patients, comparison being made with an earlier series of 253 patients who were treated with X rays.

The authors state that patients with acne simplex can usually be cured by a low fat diet, the administration of vitamin A and diethylstilbestrol and local measures. They believe that in pustular, nodular and cystic types the patients usually are sensitized to staphylococci and often harbor foci of infection which not only contribute to the pustulation and make it worse, but may be the sole cause. The staphylococci in acne patients differ in their susceptibility to antibiotics. Penicillin usually has little clinical effect and the best results have been obtained with aureomycin, terramycin and sulfadiazine. Some patients who failed to respond to these drugs were cured when dihydrostreptomycin was used.

Treatment usually consisted of several months of oral administration of diethylstilbestrol (in both girls and boys) oral administration of a suitable antibiotic or sulfonamide—terramycin being preferred, and supplementary local treatment. Sometimes the administration of the estrogen could be stopped and progress continued with antibiotics and local therapy. Some patients responded well to antibiotics and local therapy only. Topical applications of tyrothricin, bacitracin and aureomycin were sometimes beneficial.

In discussing systemic measures advisable in acne patients, it is mentioned that where the patient is under weight, has poor posture, pallor and listlessness, injections of vitamin B₁₂ or liver extract are given. These are also useful if there is much seborrheic dermatitis concomitant with the acne. When comedones are present, vitamin A is given, either orally in doses of 100,000 or 200,000 units daily or in severe cases, parenterally in the form of an aqueous solution twice weekly (in addition to the oral vitamin A).

The final results in the 384 patients treated by the antibiotic (or sulfonamide) and estrogen method are as follows: 90 patients (23%) were cured 276 (71%) were improved, and 18 (6%) were unimproved or worse. The observation period has been too short for the number of recurrences to be estimated. In the control series of 253 patients treated with X rays, the results were as follows: 152 patients (60%) were cleared up or improved 51 patients (20%) were not appreciably better or worse, and another 50 patients (20%) cleared up for a time, but then their acne returned.

*Presbyterian Hospital
New York, N. Y.*

818. AYRES, S., JR., AYRES, S., III, and MIROVICH, J. I. *Macrocytic anemia and impaired liver function in excretions and certain other dermatoses* Arch. Dermat. & Syph. 62 851-872, Dec. 1950

During the past two years the authors have encountered 27 patients with dermatoses of obscure origin in which the manifestations of macrocytic anemia found. Both the skin lesions and the blood picture

gratifying responses to intragluteal injections of crude liver extract containing folic acid, together with high potency vitamin B complex and iron administered orally. Vitamin B₁₂ was available for treatment of 4 cases. Intragluteal injection of 10 to 15 mcg. once a week has given strikingly good results.

In 83 other patients with various dermatoses, especially of an eczematous type, the liver function was impaired. In the majority of cases cure or improvement followed dietary regulation, administration of hypotrophic substances in the form of choline or methiochol[®] (which contains methionine, inositol and choline) of high potency vitamin B complex with vitamin C, of crude liver extract with folic acid given intragluteally in a dose of 2 cc. at five- to seven-day intervals, and symptomatic procedures.

Illustrative case histories are included.

Los Angeles, Calif.

- 819 DIETERICH, J. P. *Infantile eczema—therapy with vitamin B₁₂: a clinical note* Ann. West. Med. & Surg. 5 47-48, Jan. 1951

Since infantile eczema is generally considered to be of an allergic nature, and since it has been observed that a patient suffering from allergic dermatitis was affected favorably by vitamin B₁₂, the author used it in a 2 year old girl who had had eczema since she was 5 days old. Eliminating cow's milk from the diet had brought about slight improvement before vitamin B₁₂ administration was begun. The dosage of vitamin B₁₂ was 20 drops (10 mcg.) by mouth daily for one month, then 15 drops daily. Within two weeks after this treatment was started, there was a complete remission which has lasted for four months at the time of writing. The child's appetite improved, she gained in weight, and seemed to be developing more rapidly than previously.

Glendale, Calif.

820. SIMON S. W. *Vitamin B₁₂ therapy in allergy and chronic dermatoses*, J Allergy 22 183-185, March 1951

Vitamin B₁₂ therapy was evaluated in 20 adult patients with asthma. Nineteen had intractable asthma; the remaining patient had mild asthma but severe eczematoid atopic dermatitis. Four intramuscular injections of 1,000 mcg. of vitamin B₁₂ were given at weekly intervals. Treatment used prior to B₁₂ administration was continued throughout the seven-week period of study. It was found that 12 patients gained weight, 6 lost, and 2 remained the same. Vital capacity, which in all but one patient was originally below normal, showed an increase in 14, a decrease in 2, and was unchanged in 4 patients. Hematologic findings were not significantly altered. Subjectively 16 of the patients stated that there was a definite improvement with reference either to asthma, shortness of breath upon exertion, appetite, sleep, or general condition. This improvement was not always associated with an increase in vital capacity was not spectacular and could have been partly due to the institution of new treatment.

The patient who had both asthma and atopic dermatitis had a very definite and spectacular clearing of his

chronic dermatitis. This prompted further investigations with B₁₂ treatment in 28 patients with chronic dermatoses.

Ten of the patients had atopic dermatitis. 1 showed great improvement, 5 showed moderate improvement, and the remaining 4 showed none. Where there was a definite relationship between atopic dermatitis and worry fear or anxiety (neurodermatitis) no result was obtained with B₁₂ therapy.

Six of the patients had chronic contact dermatitis none of these had improved with local treatment and elimination of the causative agent, but all responded to vitamin B₁₂ with great improvement or complete clearing.

Vitamin B₁₂ was ineffective in 2 patients with dermatitis herpetiformis.

Of 10 patients with chronic urticaria lasting for one year or more, 9 were greatly improved or cured and one was definitely worse after treatment with vitamin B₁₂. The latter patient was found to have an intestinal infection. When chloramphenicol therapy was instituted, the urticaria cleared.

The authors state that no absolute conclusions can be drawn from the small number of patients treated in each category. They believe that vitamin B₁₂ is probably of no value in the treatment of chronic bronchial asthma. Further trial of vitamin B₁₂ in chronic contact dermatitis and chronic urticaria is, however, considered indicated.

Beyers, Ohio

- 821 LEVY W. *Effect of vitamin B₁₂ on ichthyosis*, Lancet 2 144, July 19 1952 (in Letters to the Editor)

"Ichthyosis is a distressing condition, so I should like to report a chance observation. A man, aged 69 recovering from a slight cerebral vascular accident, received injections of vitamin B₁₂ ('Cytasone 50') 50 µg. twice weekly, as a tonic. He has had ichthyosis all his life. After about four injections the scaling appeared to be much reduced, and after ten injections few scales are left. Is this a natural remission?"

London, N.F. 4, England

822. FRIEDMAN R., WEINER, J., and GROPAN J.: *Dermatitis herpetiformis* A.M.A. Arch. Dermat. & Syph. 64 381, Sept. 1951 (in Soc. Proc.)

A case of dermatitis herpetiformis was presented for recommendations regarding treatment. The patient had improved dramatically under sulfapyridine treatment but began to develop signs of agranulocytosis. At the time of presentation she was being treated with sulfadiazine.

Various therapeutic regimens were suggested, including vitamin B₁₂ which was reported to have been effective in one case, in doses of 1 cc. or 30 mcg.

- 823 ROSTENBERG A., JR., and PERKINS, A. J.: *Nickel and cobalt dermatitis*, J Allergy 22 466-474, Sept. 1951

A patient who was being examined for allergy to metals (later narrowed to nickel, cobalt, and gold) was found to give a delayed tuberculin type reaction to crystal

line vitamin B₁₂. The authors believe that this is the first report of an allergic reaction to crystalline vitamin B₁₂.

*University of Illinois College of Medicine and
University of Illinois College of Pharmacy
Chicago, Ill.*

- 824 ORCHARD W. E. *Dermatitis after use of penta valent arsenicals per vaginam* Brit. M. J. 2 1444 Dec. 15, 1951

A pregnant woman had an allergic reaction (dermatitis) when treated with suppositories containing penta valent arsenic for leucorrhoea. She was given promethazine hydrochloride, a vitamin B complex, and vitamin B₁₂. The acute skin condition had subsided within a week. The patient had also received penicillin and bismuth compounds for syphilis, but it was determined that neither of these chemicals were at fault in causing the dermatitis. This is a case in which a patient is sensitive to both tri valent and pentavalent arsenic.

- 825 GOLDBLATT S. *Treatment of lupus erythematosus with vitamin B₁₂ preliminary report of 4 cases*, J. Invest. Dermat. 17 303-304, Dec. 1951.

Three cases of chronic discoid lupus erythematosus responded well to intramuscular injections of 15 mcg. of vitamin B₁₂ weekly. Response was especially good in the case which was of least duration (about six months). A woman with subacute disseminated lupus erythematosus was treated with 15 mcg. of vitamin B₁₂ three times a week. Following each injection there was noticeable fading of the lesions, and they began to lose their elevation and induration. Toxic symptoms were eliminated rapidly even though the patient twice exposed herself to excessive sunlight. After one such exposure, the dosage of vitamin B₁₂ was increased to 30 mcg. after two injections the sensation of fatigue and dizziness disappeared and the erythema faded rapidly. It is considered probable that larger doses would be more effective. Doses as high as 5,000 mcg. have been reported to cause no toxic symptoms.

826. BLOCK, M. T. *Vitamin E in the treatment of diseases of the skin*, Clin. Med. 60 31-34, Jan. 1953.

In 3 patients with lupus erythematosus who responded poorly to vitamin E the lesions were cleared when vitamin B₁₂ was used, 1,000 mcg. intramuscularly twice a week for 12 doses. Two cases of leukoplakia, also, were

arrested by 1,000 mcg of vitamin B₁₂ given intramuscularly

Review, M. J.

- 827 GAUMOND E.: *Vitaminotherapie à hautes doses en dermatologie (Vitamin therapy in high dosage in dermatology)* Union méd. du Canada 82 141 155, Feb. 1953.

From published reports (about 70 references) and his personal experience the author discusses the therapeutic and pharmacodynamic effect of the various vitamins in dermatology. The results are tabulated.

In referring to vitamin B₁₂ two cases are cited, one each of lupus erythematosus and seborrheic dermatitis, in which good results were obtained with use of B₁₂ 15 mg. one to three times weekly.

Quebec, Canada

828. LEE, T. H., LERNER, A. B., and HALBERG R. J.: *Water soluble vitamins in normal human skin*, J. Invest. Dermat. 20 19-26, Jan. 1953.

This is a report of a preliminary study on the concentration of vitamin B₁₂, folic acid, riboflavin, niacin, pantothenic acid, biotin, thiamine, and ascorbic acid in the skin of 15 normal humans. Assay procedures are described in detail. The amounts found are shown in tables. In 9 samples, assayed microbiologically an average of 0.021 mcg. of water-soluble vitamin B₁₂ per Gm. of dry skin was demonstrated. The authors comment on the role of vitamins in skin as follows:

"It has been postulated that skin, like muscle, derives its energy mainly from carbohydrate metabolism. The latter is carried out by glycolysis of carbohydrate and subsequent oxidation of pyruvic acid and acetic acid, which are also reaction products of fat and protein metabolism, to the final end-products, carbon dioxide and water via Krebs' citric acid cycle. Decarboxylation of pyruvic acid to acetic acid requires a co-enzyme which is made up partly of thiamine. Furthermore, nicotinic acid is present in enzymes which catalyze several reactions in carbohydrate metabolism. Riboflavin, pantothenic acid, and biotin also play important roles in the metabolism of either carbohydrate or of protein and fat. The presence of these as well as other vitamins in considerable concentration in human skin is in support of this postulation."

*University of Michigan Medical School
Ann Arbor, Mich.*

Neoplasms

ANIMAL STUDIES

- 829 OLESON J. J., and LITTLE, P. A. *Effect of pteroylglutamic acid and vitamin B₁₂ on growth of Rous tumor implants*, Proc. Soc. Exper. Biol. & Med. 71 226-227, June 1949.

Pteroylglutamic acid has previously been found essential for the growth of implants of Rous sarcoma in young chicks. The pteroylglutamic acid can be replaced by 15-unit liver extract, or by highly purified vitamin B₁₂ concentrates. The effect of the liver extract is duplicated by as little as 0.05 mcg. a day of vitamin B₁₂. Higher levels produce larger tumors. When pteroylglutamic acid and vitamin B₁₂ are given together the tumors appear earlier and attain greater size than when corresponding levels of either factor are given alone. Thus vitamin B₁₂ does not entirely replace pteroylglutamic acid in promoting the growth of the Rous tumor but is required in addition to give maximum tumor growth under these conditions.

830. DAY P. L., PAYNE, L. D., and DINNING J. S. *Procarcinogenic effect of vitamin B₁₂ on p-dimethylaminobenzene-fed rats*, Proc. Soc. Exper. Biol. & Med. 74 834-855, Aug. 1950.

The addition of 5 mcg. of vitamin B₁₂ to a methionine-deficient diet containing 70 mg. of p-dimethylaminobenzene increased the incidence of hepatomas in rats from 17 to 78 per cent. In rats on a similar diet supplemented with methionine the incidence of hepatomas was increased only from 11 to 33 per cent by vitamin B₁₂. Methionine therefore appears to provide some protection. No tumors occurred in rats on a diet containing B₁₂ but no p-dimethylaminobenzene. Thus, there is no evidence that B₁₂ itself is carcinogenic. Its procarcinogenic effect when given with p-dimethylaminobenzene may be a reflection of its influence on metabolic transformation of the carcinogen, or may indicate that this vitamin is required for tumor growth.

University of Arkansas
Little Rock, Ark.

- 831 ENGEL, R. W., and COPELAND H. H. *The influence of dietary carotin level on tumor induction with 2-acetylaminofluorene*, Cancer Research 12 905-908, Dec. 1952.

A high (40 to 60%) casein diet gave some protection to rats fed up to 2.1 mg. per rat of the carcinogen 2-acetylaminofluorene. Addition of 90 mcg. of vitamin B₁₂ and 2 mg. of folic acid per Kg. to the diet decreased the average survival period from 42 to 32 weeks in a group of rats fed on the 60% casein diet while receiving 2.4 mg. of carcinogen per rat.

Laboratory Polytechnic Institute
Albany, N. Y.

832. SHAPIRO D. M., and GELLHORN A. *Combinations of chemical compounds in experimental cancer therapy* Cancer Research 11 85-91 Jan. 1951.

Authors' summary: "The effect of combinations of chemical compounds in cancer chemotherapy has been investigated. By the use of the guanine analog, 5-amino-7-hydroxy 1H-4-triazolo (d) pyrimidine, together with either desoxypyridoxine, pteroylglutamic acid, 7-methyl pteroylglutamic acid, or vitamin B₁₂, it has been shown that the growth of a transplantable carcinoma of the breast in mice can be inhibited to a greater extent by a combination than by any one of the drugs alone. Evidence has been presented to show that the combinations are not acting by causing host toxicity for the observed effects are associated with minimal to no weight loss. The possible mechanisms of action are discussed.

"It is recognized that in no instance did a combination eradicate the tumor being treated however the possibility of utilizing more effective agents in combination or of increasing the number of active chemical compounds in combination offers an attractive approach to achieve this end."

Columbia University College of Physicians and Surgeons
New York, N. Y.

- 833 WOOLLEY, D. W.: *Evidence for the synthesis of vitamin B₁₂ by spontaneous tumors*, Proc. Nat. Acad. Sc. 39 6-18, Jan. 1953.

Mice do not synthesize vitamin B₁₂. If a pregnant mouse ingests a sufficient amount of vitamin B₁₂, enough of this vitamin is passed on to the young to meet almost completely their needs for normal growth, even when the young are fed a B₁₂-deficient diet. This affords a means of determining whether neoplasms synthesize vitamin B₁₂. If tumor-bearing pregnant mice are kept on a B₁₂-deficient ration but their young grow normally on the deficient diet, the young must have received this vitamin from supplies manufactured by the neoplasm in the mother. This was found to be true of the offspring of Swiss mice (and, to a lesser extent, C3H and C strain mice) bearing spontaneous mammary tumors. No evidence was obtained that transplanted mammary tumors of the same strain synthesized vitamin B₁₂. This difference in metabolic behavior between normal and neoplastic tissue is recognized as "a promising pathway to chemotherapeutic attempts." It has been demonstrated previously that specific chemical compounds which are antimetabolites to dimethylaminobenzene, a precursor of vitamin B₁₂ and riboflavin, harm those living things which synthesize these vitamins but do not affect others. The synthetic ability which the tumors in these experiments possess is akin to the ability of crown galls to synthesize indole acetic acid and that of cambium tissue to form an essential growth factor which neighboring cells require but do not make. In these three instances, undifferentiated tissue differs from differentiated tissue in possessing the ability to synthesize a particular biologically important compound.

Rockefeller Institute for Medical Research
New York, N. Y.

- 834 WOOLLEY, D. W.: *Suppression of spontaneous tumors of mice through the considered use of anti-metabolites*, Science 117: 473, May 1 1953 (In Soc. Proc.)

"The study of the basis of selective action—i.e., of why a given chemical is poisonous to one living thing and harmless to another—has indicated that selective drugs can be predicted and realized through the application of existing biochemical and nutritional knowledge. Thus it can be shown that those cells which synthesize vitamin B₁₂ and riboflavin are harmed by suitable anti-metabolites, of 1,2-dimethyl-4,5-diaminobenzene, the common precursor of these vitamins. Those cells which do not conduct these syntheses and therefore exhibit a nutritional need for these vitamins, are not affected. Because spontaneous mammary tumors of mice have been found to synthesize vitamin B₁₂ in contrast to the inability of normal tissues to do likewise, the aforementioned anti-metabolites of dimethyldiaminobenzene should selectively harm the neoplasms. Three of these anti-metabolites have been shown to cause these spontaneous cancers to become smaller and occasionally to disappear. The substances are 1,2-dimethyl-4-amino-5-hydroxybenzene, 1,2-dichloro-4-(p-nitrobenzenesulfonylamido)-5-nitrobenzene, and 1,2-dimethyl-4-(p-carboxyphenylazo)-5-hydroxybenzene. Not all individuals were benefited, and, in fact, only about 50% of animals exhibited regression or disappearance of the tumors. Furthermore, the regressions of the neoplasms were not permanent. All eventually began to grow again in 1-5 months. Studies were made in an effort to ascertain why the tumors became resistant to the drugs, and why all were not originally susceptible."

*Rechercheur Associate for Medical Research
New York, N. Y.*

- 835 ENGEL, R. W.: *Influence of diet on spontaneous and nutritionally induced tumors*, Texas Rep. Biol. & Med. 10: 974-986, Winter 1952.

Author's summary "The initial observation that the prolonged feeding of diets deficient in choline promotes the production of tumors in the liver and other sites in rats has been amply confirmed by additional studies in our laboratory as well as by workers in several other laboratories. This promotion of spontaneous tumor growth through dietary restriction of choline has also been demonstrated in chickens. No tumors have been observed in dogs in the presently reported studies. The complication of early edema development was also noted to occur in rats when attempts were made to hasten the damaging action of choline-low diets by reducing the dietary protein.

"It has been pointed out that strain differences in rats may have an important bearing on the results in choline deficiency studies and that at least two strains exhibit markedly different susceptibilities to liver tumor induction by *exo* dyes. Early results indicate that the striking anticarcinogen action of riboflavin in *exo* dye carcinogenesis has its parallel in the action of riboflavin in preventing liver tumor induction by the feeding of a low-choline diet."

In the experiment with dogs it was found that 6 of the 7 on a choline deficient diet had duodenal ulcers and liver cirrhosis. Two-tenths per cent of dietary choline or a supplement of vitamin B₁₂ concentrate protected the livers of control dogs. The author points out that perhaps the high incidence of duodenal ulcers was not directly related to choline deficiency dogs on the deficient diet have variable appetites and inanition may have been a contributing factor.

*Alabama Polytechnic Institute
Tuscaloosa, Ala.*

Other Clinical and Experimental Uses

INFECTIONS

836. SAGGESE, V.: *Oral administration of living cultures of Streptomyces griseus as cure of pertussis and anemia, Minerva pediat.* 1 388, Nov 1949 (abstr. A.M.A. Am. J. Dis. Child. 83 864, March 1952)

"Excellent clinical results have been obtained by the feeding of living cultures of *Streptomyces griseus* to patients with whooping cough. Certain types of anemia were also benefited by the same treatment this is explained by the finding that the cultures contain antianemic vitamins."

837. ROBERTS, R. B., and SANDS, M.: *The influence of vitamin B₁₂ on the growth of bacteriophage T4* J. Bact. 58: 710-712, Nov 1949

This study was made to see if vitamin B₁₂ is involved in the multiplication of the virus, bacteriophage T4. Cells of the host, *Escherichia coli*, strain B, were grown in a synthetic medium. When vitamin B₁₂ was added to this medium, the burst time of the virus was decreased and larger bursts were found. It appears, therefore, that vitamin B₁₂ is one of the rate-limiting factors in the synthesis of the bacteriophage T4 by *Escherichia coli* cells in the resting phase.

The authors state that it seems probable that the B₁₂ effect on virus multiplication is through its role in thymine synthesis. They give two possible applications of their results: (1) the use of virus growth as a biological assay for vitamin B₁₂, and (2) the use of suitable B₁₂-activating or B₁₂-competing agents in virus-infected cells as means of chemotherapy

Corvallis Institution of Washington
Washington, D. C.

838. SULKIN, E. E., MANIRE, G. P., and ALLEN, R.: *The influence of vitamin B₁₂ on experimental viral infections*, Texas Rep. Biol. & Med. 8 367-368, Fall 1950 (in Soc. Proc.)

The effect of vitamin B₁₂ on the course and outcome of experimental infections with the viruses of St. Louis encephalitis and poliomyelitis (Lansing) was studied. No difference was found in mortality rates in the vitamin B₁₂-treated animals and the controls infected with poliomyelitis. Of the animals infected with the St. Louis encephalitis virus, however, the B₁₂ treated group showed increased susceptibility to this infection. In another experiment with mice infected with the St. Louis encephalitis virus, 82.5 per cent of B₁₂-treated animals were dead by the fifteenth day after inoculation, whereas only 55 per cent of the controls were dead.

Louisiana Medical School of the University of Texas
Dallas, Texas

839. MORGAN, H. R.: *Factors related to the growth of psittacosis virus (strain 68C) I Pteroylglutamic acid, vitamin B₁₂, and citrovorum factor* J. Exper. Med. 95 269-276, March 1952.

Author's summary: "The inhibitory action of sodium sulfadiazine on the growth of psittacosis virus (68C) in embryonated eggs is readily reversed by citrovorum factor but not by small amounts of vitamin B₁₂."

"In embryonated eggs, the pteroylglutamic acid analogues, 9-methylpteroylglutamic acid and 4-aminopteroylglutamic acid, produced some suppression of the growth of psittacosis virus (68C). 4-Aminopteroylglutamic acid, 4-amino-N¹⁰-methylpteroylglutamic acid, and 4-aminopteroylglutamic acid inhibited the growth of this virus in tissue cultures at concentrations which were not toxic for the host tissue. The inhibitory action of 4-amino-N¹⁰-methylpteroylglutamic acid and 4-aminopteroylglutamic acid was readily overcome by addition of citrovorum factor."

"Growth of meningopneumonitis virus in embryonated eggs or tissue culture is suppressed by 4-aminopteroylglutamic acid."

"The advantages of the tissue culture technique for studies on the growth of viruses are discussed."

University of Rochester School of Medicine and Dentistry

Rochester, N. Y.

University of Michigan School of Public Health

Ann Arbor, Mich.

840. TAMM, L., FOLKERS, K., and HORSFALL, F. L., JR.: *Inhibition of influenza virus multiplication by 2,5-dimethylbenzimidazole*, Yale J. Biol. & Med. 24 559-567 June 1952.

2,5-Dimethylbenzimidazole, the 5,6-isomer of which is a moiety of vitamin B₁₂, is of interest in regard to possible selective inhibitory activity on viral multiplication. Experiments are reported which indicate that this compound inhibits multiplication of influenza viruses through an effect upon the host cell rather than on the virus. Multiplication of influenza B virus (Lee strain) or influenza A virus (PR8 strain) in the chorioallantoic membrane in vitro is inhibited even when dimethylbenzimidazole is given long enough after inoculation so that the process of intracellular multiplication of the agent is well under way. The compound does not inactivate the virus nor prevent its adsorption by the membrane, and the membrane retains its capacity to support viral multiplication. Oxygen consumption of the membrane is not affected. Prolonged exposure of the membrane to dimethylbenzimidazole does not diminish the capacity of the membrane to support multiplication of the Lee virus.

Harvard Medical School, Department of Medical Research

New York, N. Y.

March 4, 1952

Rechnung H. J. Jan.

841. WERTMAN, K., and SARANDRIA, J. L.: *Complement fixing murine typhus antibodies in vitamin deficiency states IV B₁₂ deficiency* Proc. Soc. Exper. Biol. & Med. 81: 395-397 Nov 1952.

The effect of vitamin B₁₂ deficiency on the production of complement fixing antibody for murine typhus rickettsiae was studied in 58 rats in an attempt to explain the increased susceptibility and mortality in malnourished populations exposed to infection.

Authors summary: "This study indicates that a vit. B₁₂ deficiency impaired the production of circulating complement-fixing antibody. The sera of the inanition control and the ad libitum control animals possessed antibody concentrations of approximately the same titers. This was evident when either 0.0073 mg. N or 0.0365 mg. N was employed as the antigen. Therefore, the specific vitamin deficiency and not inanition appears to be the significant factor affecting the production of circulating complement-fixing antibody under these experimental conditions."

University of Pittsburgh
Pittsburgh, Pa.

842. MAY C. D., STEWART C. T., HAMILTON A., and SALMON R. J. *Infection as cause of folic acid deficiency and megaloblastic anemia, AJMA. Am. J. Dis. Child.* 94: 718-728, Dec. 1952.

Authors summary: "The spontaneous occurrence of megaloblastic anemia in association with infection in infants and in monkeys is described."

"Megaloblastic anemia was induced experimentally in monkeys by producing abscesses with intramuscular injections of turpentine."

"Analyses of the liver for ascorbic acid, vitamin B₁₂ and folic acid compounds in natural and experimental infections are presented."

"The low content of folic acid compounds in the liver in both natural and experimental infections and the elimination of megaloblastosis from the marrow by folic acid, but not by vitamin B₁₂ or ascorbic acid, leads to the conclusion that infection can cause a deficiency of folic acid compounds."

"The application of these observations to the various types of megaloblastic anemia seen in infants and to treatment is discussed."

Emeryville, N. H.

ASTHMA

843. KAUFMAN, R. E. *Effect of vitamin B₁₂ in asthma, Ann. Allergy* 9: 517-518, July-Aug. 1951.

In only one patient, of a small series of 8, was there improvement under vitamin B₁₂ therapy

Longsight Hospital
New York, N. Y.

844. POTTINGER, F. M., JR., and KROHN B. *Emergency treatment of the asthmatic with special reference to adrenal cortex and vitamin B₁₂, Rocky Mountain M. J.* 49: 583-586, July 1952.

The authors discuss emergency treatment of the asthmatic patient, which aims at correcting the disturbances resulting from adrenal exhaustion, and includes use of the adrenal hormones and vitamin B₁₂. There are 2 case histories. The first patient, a 62 year old man, received intravenous adrenal cortical extract, 250 dog units, and 60 mcg. of vitamin B₁₂ for immediate relief of distress. His regimen included oral adrenal cortical extract from 10 Gm. of gland, and salt, three times daily. A recurrence of asthma over a month later was treated with

300 mg. of cortisone daily gradually reduced to 50 mg. over two weeks. For two succeeding attacks he received corticotropin, 80 mg. daily initially gradually reduced to 20 mg. daily. Corticotropin was given rather than cortisone because the patient's blood pressure had risen. The authors felt that cortisone and corticotropin did not produce any better results than the smaller doses of crude adrenal extract. The second patient, a 67 year old woman, received intramuscularly 250 dog units of adrenal cortical extract daily for 10 days, and responded well.

Another attack a year later was treated successfully with adrenal cortical extract, 500 dog units, and 60 mcg. of vitamin B₁₂. In general, adrenal cortical extract in a dose of 50 to 250 dog units is helpful in stopping the attack, and may also combat coincident respiratory infection. The most severe cases are helped by an intravenous drip containing adrenalin, 500 to 1,000 dog units of crude adrenal extract (depending on degree of exhaustion of the patient), 60 mcg. of vitamin B₁₂, and calcium gluconate. Besides its hemopoietic action, which aids oxygenation, B₁₂ appears to extend the action of the adrenal cortical hormones, and also promotes lipid metabolism, thus contributing to the general welfare of the patient.

Neurosis, Calif.

845. CARUSELLI, M. *Therapy of asthma with vitamin B₁₂, Riforma med.* 66: 849- Aug. 2, 1952 (abstr. J.A.M.A. 150: 1731, Dec. 27 1952)

"Good results were obtained with vitamin B₁₂ in 10 of 12 patients with asthma to whom the vitamin was given daily in doses of 30 mg. for 15 to 20 days. In six of the patients, the disease was due to bacterial allergy. Symptoms improved, the asthmatic attacks ceased, and there was a pronounced improvement in general condition, with weight gains. This therapy also cured severe constipation in one patient and dyspepsia due to hyperacidity in another. An antiallergic action of the vitamin is suggested. A recurrence in two patients after three and eight months, respectively subsided when the vitamin was given again. Two patients in whom asthma was associated with circulatory decompensation and whose condition was aggravated by strophanthin and digitalis improved with vitamin B₁₂. This result may be due to an improvement in hemodynamics and oxygenation of the blood or more likely to a direct action of the vitamin on the nutrition and function of the myocardium. The two patients in whom this therapy failed were old persons in whom asthma was associated with severe pulmonary emphysema and hypertensive arteriosclerosis. In these patients the disease may no longer have been due to active allergic factors but to irreversible anatomic and functional changes."

BLOOD AND CARDIOVASCULAR

846. BARNARD R. D.: *Indiscriminate transfusion, a critique of case reports illustrating hypersensitivity reactions, New York State J. Med.* 51: 2399-2402, Oct. 15, 1951.

The author reports 4 cases in which the patients were hypersensitive to blood transfusions. Three of the patients died as a result of transfusion. In one of these patients who was leukemic, animal protein factor and terra

Author's summary: "1) These data show that B₁₂ increased the tensile strength of wounds during the early phases of healing in rats fed balanced diets containing 19% or 25% protein. This effect was evident at least by the third day in the wounds studied, and it was most noticeable by the sixth day. 2) The wounds that were studied in the saline control and vitamin treated rats from the third to eighth day showed no significant difference in their healing rates at the eighth day. 3) The delayed healing rate and increased incidence of wound infection in rats that were protein depleted by diet were not significantly altered by administration of vit. B₁₂."

Columbia University College of Physicians and Surgeons, and
Franklin D. Roosevelt Hospital
New York, N. Y.

856. FINDLAY C. W., JR.: *Vitamin B₁₂ accelerates wound healing in rats*, Mod. Med. 21: 131, July 1, 1953 (in Late Reports from Medical Centers)

"Vitamin B₁₂ accelerates wound healing in rats, especially in early stages, probably by taking part in protein synthesis. With a fair to good supply of dietary protein, tensile strength of wounds was increased at least by the third postoperative day and notably by the sixth. Dr. Charles W. Findlay Jr., produced as good results with treatment after surgery as when preoperative doses were also given. Healing did not improve in animals on a low protein diet."

Columbia University
New York, N. Y.

General

- 857 ELVERHEM, C. A.: *The vitamin B complex*, J.A.M.A. 138 960-971 Nov. 27, 1938.

A revision of chapter 11 in the first "Handbook of Nutrition" presents excellent reviews of the factors of the vitamin B complex, including B₁₂ (p. 971).

858. ANNOTATION: *Further studies on vitamin B₁₂*, Nutrition Rev. 6 291-293, Oct. 1948.

- 859 DAMESHEK, W.: *And now B₁₂!* Blood 4: 76-78, Jan. 1949 (Editorial).

Commenting on the extraordinary potency of vitamin B₁₂, the author expresses himself as follows: "In these crowded days when one therapeutic miracle succeeds another in rapid succession, the appearance of a new substance with almost incredible therapeutic effects inspires but little excitement. The isolation of vitamin B₁₂ in the research laboratories of Merck and Company in this country and almost simultaneously in the Glaxo Laboratories in England is the most recent case in point. Here is a substance that, when given to a patient suffering from pernicious anemia, results in a maximal reticulocyte response following a single injection of 5 to 10 thousandth of a milligram (0.000005 Gm.)! Has there ever been in the history of medicine a more potent material, microgram for microgram?"

Comparison is made between vitamin B₁₂, folic acid, and liver extract in the treatment of the various anemias, but at the time the article was written any conclusions were necessarily tentative. Evidence from the literature is presented which suggests the identity of vitamin B₁₂ with the "animal protein factor" present in crude materials such as fish meal, cow manure, and liver.

860. AMYOT, R.: *La vitamine B₁₂ serait-elle la principale substance antianémique des extraits de foie?* (Is vitamin B₁₂ the main antianemic substance in liver extract?) Union méd. du Canada 78 64-65, Jan. 1949.

The author reviews the work done on vitamin B₁₂ and concludes that this vitamin might be the only active substance in liver extract.

861. WOODS, R.: *The story of vitamin B₁₂*, Borden's Rev. of Nutr. Res. 10 112, Jan. 1949.

In this review article, progress in treatment of pernicious anemia is traced, beginning in the 1920's when it was first recognized that the disease was a manifestation of vitamin deficiency. Contributions of the numerous clinicians and other investigators leading to the isolation and identification of vitamin B₁₂ are discussed.

862. LEADING ARTICLE: *Vitamin B₁₂*, Lancet 1 151-152, Jan. 22, 1949.

This editorial reviews the literature on vitamin B₁₂ and concludes with the following sentence: "Meanwhile [until the chemical constitution of vitamin B₁₂ is known and its properties established] the practitioner will be

well advised to keep to the recognized methods of treatment—with liver extracts that have been clinically before, or if treatment by mouth is preferred, a dedicated stomach."

863. EDITORIAL: *Vitamin B₁₂*, Minnesota Med. 3 June 1949.

864. ANNOTATION: *Oral B₁₂ seen as possibility*, Trade News 24 35, Aug. 8, 1949.

Investigations conducted by workers of the City of Michigan Hospital indicate that an extract containing may cause utilization of orally administered B₁₂. This effect is thought to be identical with B₁₂ potentiating effect of normal human gastric Vitamin B₁₂ and Castle's "extrinsic" factor are likely or closely related.

865. LEADING ARTICLE: *Progress with vitamin B₁₂*, Oct. 2 565-566, Sept. 24, 1949.

Review article.

866. TASTALDI, H.: *Fator antianêmico do fígado* (The liver factor and vitamin B₁₂), Arq. Biol. (São Paulo) 33 181-182, 1949.

A comprehensive review on the liver factor as vitamin B₁₂ with 149 references.

867. CORDIER, R.: *Contribution à l'étude de la vitamine B₁₂ (à propos de 9 observations personnelles)* (Contribution to the study of vitamin B₁₂ [personal observations]), Thèse, Imprimerie Beaux-Arts, Lyon, 1949 (review Presse méd. 76, Jan. 25, 1950).

Accounts of the first treatments with vitamin B₁₂ in France are given (in the original, not in the review). Indications, criteria of action, and dosage of vitamin are discussed. The importance of preventing recidivism by injections of 10 mcg. of vitamin B₁₂ at intervals determined by the individual case is stressed. The active use of gastric juice or acidification of the contents of the stomach to increase the action of vitamin is mentioned. Sources of vitamin B₁₂ other than liver are discussed.

868. GIRDWOOD, R. H.: *Vitamin B₁₂ and related: a clinical and experimental review*, Edinb. M. J. 57 72-109 Feb. 1950 (abstr. Blood Jan. 1951).

"This review mentions work on the liver, extrinsic and intrinsic factor, yeast and yeast extracts and folic acid. Animal factors related to vitamin are discussed; also methods of assay and procedure in isolation of B₁₂.

"Animal experiments designed to produce a pernicious anemia are reviewed with their response to pyridoxine and B₁₂. The clinical application of vitamin B₁₂ is described.

Part II
Analytical Studies of Vitamin B₁₂

Analytical Studies

VITAMIN B₁₂: IDENTIFICATION ISOLATION

891. SHORR, M. S.: *Unidentified essential growth factors for Lactobacillus lactis found in refined liver extracts and in certain natural material*, J. Bact. 53 669 May 1947 (in Soc. Proc.)

Lactobacillus lactis failed to grow in an amino acid basal medium containing all of the synthetic B vitamins or when the medium was supplemented with either clarified tomato juice or certain preparations of liver extracts, but grew when the tomato juice and liver preparations were added together. An assay method was developed for each factor. The liver factor was found in high concentrations in refined liver extracts and in lower amounts in Wilson liver fraction L, brewers yeast, liquid skim milk, peptin, erepsin, unclarified tomato juice, bacto yeast extract, autolyzed yeast, trypsin, peptone, tryptone, tryptose, and peptonized milk. These materials had some tomato juice factor activity. Crude casein, calcium caseinate, alcohol-extracted casein, acid-hydrolyzed "vitamin free" casein, tryptic digest of crude or "vitamin-free" casein, zinc insulin crystals, and chymotrypsin were inactive for the liver factor but had tomato-juice factor activity. Inulin, chymotrypsin, and some tomato juices also showed some inhibitory action. Phosphopentose, ascorbic acid, yeast, nucleic acid, sodium salt of thymonucleic acid, and adenylc acid were inactive for both factors. A third factor was synthesized. The liver factor does not appear to be identical with any of the unidentified growth factors for bacteria in distribution and properties. The other factor(s) may be related to certain bacterial and animal growth factors.

U. S. Department of Agriculture
Washington, D. C.

892. SHORR, M. S.: *Unidentified growth factors for Lactobacillus lactis in refined liver extracts* J. Biol. Chem. 169 455-456, July 1947

It has been found that *Lactobacillus lactis* Dorner requires two unidentified factors for growth in an amino acid basal medium containing all the synthetic B vitamins (J. Bact. 53 669 May 1947 [Abstr. 891]). One factor (TJ) is present in clarified canned tomato juice and, in low amounts, in casein, as well as in many other substances. The second (LLD) is found in high concentrations in liver extracts which are active for rat growth, but not in casein or casein hydrolysates. Assays for the LLD factor in crude and refined liver extracts of the type used for intramuscular injection in the treatment of pernicious anemia show that the LLD factor is concentrated in the refined extracts in almost linear relationship to the potency of these extracts in pernicious anemia. This suggests that the LLD factor may be the principle which acts against this disease. The TJ factor is usually more concentrated in crude than in refined liver extracts, and may or may not be active in the treatment of anemia. A third factor appears to be synthesized by *L. lactis* Dorner. Inhibitory substances are present in many natural materials. *L. lactis* Dorner should be of value in the study of these factors and their relation to anemia.

893. RICKES, E. L., BRINK, N. G., KONIUSZY, F. R., WOOD, T. R., and FOLKERS, K.: *Crystalline vitamin B₁₂*, Science 107 396-397 April 16, 1948.

A crystalline compound, tentatively designated as vitamin B₁₂, which in microgram quantities produces a positive hematologic response in Addisonian pernicious anemia, has been isolated from liver. Dr. Mary S. Shorr, at the University of Maryland, found that the microorganism *Lactobacillus lactis* Dorner required two unidentified growth factors one of which appeared to be related to the activity of commercial liver preparations used in the treatment of pernicious anemia. (Abstr. 891, 892.) Further work on the purification of liver concentrates, which had been in progress at the Merck Research Laboratories for some time, led to the isolation of a crystalline compound which was highly active for the growth of *L. lactis* (LLD factor). Compared with an arbitrarily selected standard liver concentrate assigned a potency of 1,000 LLD units per mg., the new compound, which crystallizes in the form of minute red needles, has a potency of about 11,000,000 LLD units per mg.

Preliminary clinical tests indicate that vitamin B₁₂ has extremely high biologic activity. A single intramuscular dose of 150 micrograms produced a very strong hemopoietic response in 1 patient with pernicious anemia, and doses of 3 and 6 micrograms, respectively, induced a prompt increase in circulating reticulocytes, red cells, and hemoglobin in 2 other pernicious anemia patients. If this compound is the only therapeutically active substance present in commercial liver extracts, clinical and hematologic responses should be obtained from the parenteral administration of about 1 microgram of vitamin B₁₂ daily. Approximate vitamin B₁₂ contents of several commercial liver extracts for parenteral use are compared.

VITAMIN B ₁₂ CONTENT (Graham-Smith Assay)				
Liver extract	LD ₅₀ units/mg.	mg./ml.	mg./T. 2.5 ml.	% dry weight
Company A	72,000	6.5	0.1	0.006
Company B				
Sample 1	13,000	1.2	0.1	0.0065
Sample 2	20,000	1.7	0.1	
Company C				
Sample 1	124,000	12.0	0.8	0.014
Sample 2	90,000	8.0	0.8	0.010
Company D				
Sample 1	57,000	2.6	0.2	0.0071
Sample 2	50,000	2.3	0.2	0.0071

894. SMITH, E. L.: *Purification of anti-pernicious anemia factors from liver* Nature 161 633-639 April 24, 1948 (Letter to Editor)

Two red pigments, both highly active in pernicious anemia, have been isolated from ox liver and from a commercial proteolyzed liver extract. More than 80 clinical tests were made during the fractionation procedures. Activity and color proved to be inseparable. The minimum effective single dose of the nonproteolyzed liver substance has been estimated at about 0.6 mg., while

protoyzed liver yielded a fraction effective at 0.3 mg., although this was not fully purified (since only 25 per cent of the original clinical activity was recovered). The latter fraction was effective in 3 cases of subacute combined degeneration of the spinal cord. Ten batches of red materials all proved clinically active in pernicious anemia (26 cases in all). Further purification of the protoyzed liver fraction has yielded a product which has eight times its intensity of color. This substance has not yet been tested clinically, but if activity remains proportional to color it should be effective at 0.04 mg. The characteristics of the substances are described. It is concluded that they are differing forms of the classical liver factor originally postulated by Minot and Murphy (*Addendum*. After this letter was written, the writer was informed of the isolation of crystalline vitamin B₁₂ by Merck & Company investigators.)

895. SMITH, E. L., and PARKER, L. F. J. *Purification of anti-pernicious anemia factor*. *Biochem. J.* 43 viii, May 29 1948 (in Soc. Proc.)

896. ANNOTATION: *Anti-anemic substances from liver*. *Lancet* 1 876, June 5, 1948.

Mr. E. Lester Smith, of Glaxo Laboratories, has crystallized the anti-pernicious anemia factor which has been isolated from liver. In a report to the Biochemical Society he said that the crystals obtained resemble those of vitamin B₁₂ as illustrated by American workers, and that calculations from color intensity suggest a minimum effective dose of the same order.

897. SMITH, E. L., and PARKER, L. F. J.: *Vitamin B₁₂ isolated in England*, *Chem. & Eng. News* 26 2218, July 26, 1948.

Shortly after the announcement in the United States of the discovery of an anti-pernicious anemia factor called vitamin B₁₂ by Merck & Co., Inc., a similar announcement was made by Glaxo Laboratories, Ltd., of England. The English workers reported at a meeting of the Biochemical Society at Oxford that they had concentrated about 1 Gm. of the product from 4 tons of ox liver by means of partition chromatography. Red crystals very similar to those exhibited by the Merck group were obtained.

898. RICKES, E. L., BRINK, N. G., KONTUSZY, F. R., WOOD, T. R., and FOLKERS, K. *Comparative data on vitamin B₁₂ from liver and from a new source, Streptomyces griseus*, *Science* 108: 634-635, Dec. 3, 1948.

Milk powder, beef extract, and culture broths of strains of *Mycobacterium smegmatis*, *Lactobacillus arabinosus*, *Bacillus subtilis*, and of several *Streptomyces* species (*S. roseochromogenus*, *S. griseus*, and *S. antibioticus*) were found to promote the growth of *Lactobacillus lactis*. The properties of a red crystalline compound isolated from one of these sources, a grisein-producing strain of *S. griseus*, were found on test to be similar to those reported for vitamin B₁₂. Like vitamin B₁₂, the crystals contain cobalt and phosphorus. They have comparable activity for the growth of *Lactobacillus lactis*, and show "animal protein factor" activity for the chick equal to that of vitamin B₁₂. Randolph West has

tested the crystals clinically (personal communication) and has found that the response in pernicious anemia parallels that obtained with vitamin B₁₂. These findings are evidence that the crystals from the microbiologic source and vitamin B₁₂ are identical.

899. ELLIS, B., PETROW, V., and SNOOK, G. F. *The isolation of the crystalline anti-pernicious anemia factor from liver*. *J. Pharm. and Pharmacol.* 1 60-61 Jan. 1949.

900. SMITH, E. L. *Crystalline anti-pernicious anemia factor*. *Brit. M. J.* 2 1367 1369 Dec. 17 1949.

This is a review of work that led to the isolation of crystalline vitamin B₁₂ at Glaxo Laboratories, with comment upon the value of the vitamin for standardization of liver preparations, the chemical nature of B₁₂, and production of a purified concentrate from *Streptomyces griseus* fermentation liquors by the Merck group.

Glaxo Laboratories, Ltd., Greenford, Middlesex, England

901. FANTES, K. H., PAGE, J. E., PARKER, L. F. J., and SMITH, E. L. *Crystalline anti-pernicious anemia factor from liver*. *Proc. Roy. Soc.* 136 592-613, Jan. 1950.

A red crystalline substance has been isolated in a very small yield from ox liver; it has been shown to have intense activity against pernicious anemia. It is probably identical with vitamin B₁₂ isolated a few weeks previously in America. The procedures by which it was isolated are reported, as are the results of a number of physical measurements and chemical analyses of the substance.

A second factor which is also active clinically and microbiologically has been separated, but has not yet been crystallized.

From clinical experiments it appears that the minimum effective single dose in pernicious anemia is 10 mcg. or even only 8 mcg. of the crystalline factor, with a daily requirement of about 0.5 mcg. It is evident that the anti-pernicious anemia factor is more potent than any known vitamin or hormone. This substance is able to check and, to some extent, reverse the neurologic sequelae of pernicious anemia to the same extent as crude liver extract. It is suggested that the crystalline anti-pernicious anemia factor together with the other red factor may be the only effective antianemic substances present in refined liver extract.

Glaxo Laboratories, Ltd., Greenford, Middlesex, England

902. HODGKIN, D., PORTER, M. W., and SPILLER, R. C. *Crystallographic measurements on the anti-pernicious anemia factor*. *Proc. Roy. Soc.* 136 609-613, Jan. 1950.

Five different samples of the crystalline anti-pernicious anemia factor were examined by various crystallographic techniques. They were shown to contain the same molecular structure. The morphology of the crystals is described and certain optical measurements are given. From X-ray data it was determined that the molecular weight of the anti-pernicious anemia factor is between 1360 and 1575.

University of Oxford, Oxford, England

CHARACTERIZATION

903. SMITH E. L.: *Presence of cobalt in the anti-pernicious anemia factor* *Nature* 162 144-145 July 24, 1948 (Letter to Editor)

The presence of cobalt has been detected in the anti-pernicious anemia factor recently isolated by Glaxo Laboratories by colorimetric estimation, crystals dried in vacuo at 56 C. contained 4 per cent. If each molecule contains one atom of cobalt, the molecular weight of the compound, allowing for 8 per cent loss on drying, is 1,600. The molecular weight of air-dried material by X-ray crystallography is 1,550 to 1,750 (the amount of available material was inadequate for accurate determination of density). The value by the diffusion method is about 3,000. Cobalt has been shown to be effective in a condition involving anemia in ruminants feeding on pastures deficient in this essential trace element. Analytical figures indicate that the molecule also contains three atoms of phosphorus.

904. RICKES, E. L., BRINK, N. G., KONTUSZY F. R., WOOD T. R., and FOLKERS K.: *Vitamin B₁₂ as cobalt complex*, *Science* 108 134, Aug. 6, 1948.

Spectrographic analysis of Vitamin B₁₂ has revealed the presence of cobalt. The vitamin appears to be a cobalt coordination complex which, having six groups about the cobalt atom, could involve one or more organic moieties. The red color of B₁₂ is apparently associated, at least in part, with its cobalt-complex character. Cobaltous ion (1 mcg./cc.) had no activity for *Lactobacillus lactis*, for which B₁₂ is a highly potent growth factor 0.000013 mcg./cc. promoting half maximum growth. It was also ineffective in 2 cases of pernicious anemia although it was given in excess of the estimated average adult daily dietary intake of cobalt. Phosphorus is also present in B₁₂, as well as nitrogen, although tests for sulfur have been negative.

Microbiologic assay has shown that B₁₂ in aqueous solution withstands autoclaving for fifteen minutes at 121 C., that in sodium hydroxide solution at room temperature it is inactivated 20 to 95 per cent in 40 minutes to 95 hours, and that in hydrochloric acid solution at room temperature it is inactivated 18 to 99 per cent in 3 to 95 hours.

905. BRINK, N. G., WOLF D. E., KACZKA, E., RICKES E. L., KONTUSZY F. R., WOOD T. R., and FOLKERS K.: *Vitamin B₁₂. IV Further characterization of vitamin B₁₂*, *J. Am. Chem. Soc.* 71 1854-1856, May 1949

Further observations on the physical and chemical characterization of vitamin B₁₂ are described. The molecule of vitamin B₁₂ possesses one cobalt atom and one phosphorus atom. Vitamin B₁₂ is levorotatory. The molecule is not a peptide, since hydrolysis of the vitamin does not liberate α -amino acids. Alkali fusion of vitamin B₁₂ forms products which react with p-dimethylaminobenzaldehyde characteristic of certain cyclic five-membered nitrogen-containing compounds including pyrroles.

906. ELLIS B., PETROW V., and SNOOK, G. F.: *The chemistry of antipernicious anemia factors. Part I. The liberation of phosphorus as phosphate from vitamin B₁₂*, *J. Pharm. and Pharmacol.* 1 237-291 May 1949

It has been found that acid hydrolyzates of vitamin B₁₂ contain phosphate. The atomic ratio of Co P in B₁₂ is 1:1. From a comparison of this proportion with the values recorded for the anti-pernicious anemia factor of Smith and Parker it is concluded that this factor is not identical with vitamin B₁₂.

907. SMITH, E. L.: *Vitamin B₁₂*, *J. Pharm. and Pharmacol.* 1 500, July 1949

The crystalline anti-pernicious anemia factor isolated by Glaxo workers, and vitamin B₁₂ isolated by Merck workers, are identical in the following respects: cobalt and phosphorus content, microbiological activity, ultra violet and visible absorption spectra; single crystal x-ray diagrams, refractive indices, polarographic step behavior on paper chromatograms; clinical potency.

908. CUTHBERTSON W. F. J.: *Biochemistry 2 Haemopoietic factors (Folic acid and vitamin B₁₂)*, *Ann. Rep. Chem. Soc.* 46 229-244, 1949

Review article 184 references.

909. SMITH, E. L.: *Folic acid, vitamin B₁₂, and anaemia. I. Chemical aspects*, *J. Pharm. and Pharmacol.* 2 409-417 1950.

Review article.

910. CHAIET L., ROSENBLUM, C., and WOODBURY D. T.: *Biosynthesis of radioactive vitamin B₁₂ containing cobalt-60*, *Science* 111 601-602, June 2, 1950.

The authors report the preparation of highly pure, crystalline, radioactive vitamin B₁₂ by the addition of radioactive cobalt-60 sulfate to a fermentation broth in occluded with *Streptococcus griseus*.

Harvard & Co., Inc., Cambridge, Mass.

911. SMITH, E. L.: *Vitamin B₁₂*, *Lancet* 2 407 Sept. 23, 1950 (In Soc. Proc.)

At the International Congress of Hematology it was reported that the molecular weight of vitamin B₁₂ is now estimated at 1650 \pm 100. Various parts of the molecule have been identified but much of it, including the site of cobalt attachment, remains unknown. Three other clinically active related factors have been identified, but their chemical differences have not been established.

912. BRINK, N. G., KUEHL, F. A., JR., and FOLKERS K.: *Vitamin B₁₂, the identification of vitamin B₁₂ as a cyano-cobalt coordination complex*, *Science* 112 354, Sept. 29 1950.

The authors have found that vitamin B₁₂ contains one cyano group bound coordinatively to the cobalt.

Vitamin B_{12} does not contain this group. Vitamins B_{12a} and B_{12b} are apparently identical. Since vitamin B_{12} is not toxic, the cyano group must be tightly bound within the coordination complex. Doses as large as 1600 mg./Kg. of body weight, given both intraperitoneally and intravenously to mice, produced no deaths or toxic symptoms. This dose level corresponds to 112,000,000 times the daily human dose of 1 mcg. of vitamin B_{12} listed in the U S Pharmacopeia.

Merck & Co., Inc.
Rahway, N. J.

- 913 BEAVER G. H., HOLIDAY E. R., JOHNSON E. A., ELLIS B., and PETROW V. *A mode of linkage of component γ in vitamin B_{12}* , J. Pharm. and Pharmacol. 2 753, Oct. 1950 (in Letters to the Editor)

In addition to absorption spectra studies, reference is made to the action of cyanide on the spectrum of B_{12} and the conclusion that "the reversible formation of a cyanide complex is dependent upon co-ordination of cyanide ion with cobalt."

London, England

- 914 BEILER, J. M., MOSS J. N., and MARTIN G. J. *Formation of a competitive antagonist of vitamin B_{12} by oxidation*, Science 114 122-123, Aug. 3 1951.

Treatment of vitamin B_{12} in a strong acid solution with hydrogen peroxide was found to form a reaction product that was competitively antagonistic to B_{12} . To 10 cc. of B_{12} solution was added 5 cc. of concentrated HCl. A few drops of a 30 percent solution of hydrogen peroxide was then added and the solution was stirred at room temperature. The solution decolorized, and after standing an hour was neutralized with sodium hydroxide. When the activity was assayed on microorganisms, it was shown to have an inhibitory effect that could be counteracted by B_{12} . When tested on organisms that do not need preformed vitamin B_{12} , no inhibitory effects were observed. The chemical structure of the antagonist is not known, but the cyanide-cobalt complex of B_{12} was not being attacked.

The National Drug Co.
Philadelphia, Pa.

- 915 DAVIS, R. D. *Aromatic biosynthesis. III Role of p-nitrobenzoic acid in the formation of vitamin B_{12}* , J. Bact. 62 221-230, Aug. 1951.
- 916 ALICINO J. F. *Perchloric acid salt of vitamin B_{12}* , J. Am. Chem. Soc. 73 4051, Aug. 1951.
- Its composition seems to be $C_{62}H_{84}N_{14}O_{18}PCl_6$.

GHCO₄.

DEGRADATION, SYNTHESIS

- 917 BRINK, N. G., and FOLKERS, K. *Vitamin B_{12} 5,6-Dimethylbenzimidazole a degradation product of vitamin B_{12}* , J. Am. Chem. Soc. 71 2951, Aug. 1949 (Letter to Editor)

Degradation of vitamin B_{12} by acid hydrolysis has given a new basic compound which has been identified by its reactions and by synthesis as 5,6-dimethylbenzimidazole.

- 918 ELLIS, B., PETROW V., and SNOOK, G. F. *The "ninkhydrin-reacting" hydrolytic fragment of vitamin B_{12}* , J. Pharm. and Pharmacol. 1 735, Oct. 1949

The present study indicates that the "ninkhydrin-reacting" substance reported earlier is probably 2-amino-3-propanol.

- 919 HOLIDAY E. R., and PETROW V. *Vitamin B_{12} as a 5,6-dimethylbenzimidazole derivative*, J. Pharm. and Pharmacol. 1 734-735, Oct. 1949

Hydrolytic fragments of vitamin B_{12} have been identified with 5,6-dimethylbenzimidazoles.

National Research Spectrographic Unit, and
Doubleday Drug Stores
England

- 920 BEAVER G. H., HOLIDAY E. R., JOHNSON E. A., ELLIS, B., MAMALIS, P., PETROW V., and STURGEON B. *The chemistry of anti-pernicious anemia factors. Part III 5,6-disubstituted benzimidazoles as products of acid hydrolysis of vitamin B_{12}* , J. Pharm. and Pharmacol. 1 957 970 Dec. 1949

The presence of three chemically related substances (components α , β and γ) in vitamin B_{12} acid hydrolyzates has been established. Components α and β are 1,5,6-benzimidazoles and γ a 5,6-disubstituted (5,6-dimethylbenzimidazole). On acid hydrolysis only one 5,6-dimethylbenzimidazole nucleus is released from B_{12} . The benzimidazole nucleus exists preformed in the vitamin and α , β , γ represent successive stages of degradation of a common precursor. The biogenesis and structure of B_{12} is discussed.

- 921 ELLIS, B., PETROW V., and SNOOK, G. F. *The chemistry of anti-pernicious anemia factors. Part II The "ninkhydrin-reacting" hydrolytic fragment of vitamin B_{12}* , J. Pharm. and Pharmacol. 1 950-956, Dec. 1949

Earlier findings that hydrolysis of vitamin B_{12} yields a "ninkhydrin-reacting" substance have been confirmed. This substance is identical with 2-amino-3-propanol. Evidence has been obtained for the presence in acid hydrolyzates of vitamin B_{12} of material showing selective adsorption with bands and inflections at 2850, 2768, 2690, 2585 and 2500 A (referred to as "the 285 component"). This "285 component" is resolved into at least two structurally related substances by chromatography employing n-butyl alcohol-acetic acid as the irrigation solvent. Formation of ammoniac acid occurs during acid or alkaline hydrolysis of vitamin B_{12} .

- 922 BRINK, N. G., HOLLY F. W., SHUNK, C. H., FEEL, E. W., CAHILL, J. J., and FOLKERS, K. *Vitamin B_{12} IX 1-q-D-Ribofuranoside-5,6-dimethylbenzimidazole a degradation product of vitamin B_{12}* , J. Am. Chem. Soc. 72 1866, April 1950 (Communication to the Editor)

1-q-D-Ribofuranoside-5,6-dimethylbenzimidazole has been obtained by degradation of vitamin B_{12} and by

synthesis. For convenience the names α and β ribazole have been designated for the corresponding 1 D ribofuranoside-5-g-dimethylbenzimidazoles.

Smith & Co., Inc.
Baltimore, Md.

923. WOLF D. E., JONES W. H., VALIANT J., and FOLKERS, K.: Vitamin B₁₂. XI Degradation of vitamin B₁₂ to D,L-amino-2-propanol, J. Am. Chem. Soc. 72 2820, June 1950 (Communication to the Editor)

Degradation of vitamin B₁₂ by acid hydrolysis has yielded D,L-amino-2-propanol which was characterized by structure examination and by synthesis.

Smith & Co., Inc.
Baltimore, Md.

924. BUCHANAN J. G., JOHNSON A. W., MILLS J. A., and TODD A. R.: The isolation of a phosphorus-containing degradation product from vitamin B₁₂ and B_{12a}, Chem. & Indust., p. 426, June 3, 1950.

Studies of the acid hydrolysis (a-h) of vitamin B₁₂ and vitamin B_{12a} are reported. B_{12a} is the "unmethylated factor" (Vitamin B₂ mentioned by E. Lester Smith *et al.*). Its approximate formula is C₅₄H₈₄O₂₂N₁₄PCo and it is biologically and clinically similar to B₁₂. The difference between B₁₂ and B_{12a} lies in the cobalt-containing colored fraction, since the colorless a-h products from both are identical H₂PO₄ NH₄Cl; aliphatic reacting base varies 5,6-dimethylbenzimidazoles (I) the nature of which depends upon the conditions of a-h. Moderate a-h gives a-N-substituted I (II) stronger a-h gives II and β -N substituted I (III) and still more vigorous a-h yields I itself. II contains the basic nucleus represented by I substituted in the 1-position by a phosphorylated side chain (5 or 6 C atoms with several OH groups). III is diphosphorylated II. Both II and III are glucosides. The phosphoryl group is attached to C₅ or C₆ in the sugar residue. It is suggested that B₁₂ and B_{12a} are trisubstituted derivatives of phosphoric acid, the base and colored fragment being attached to the I nucleotide (II) through the two free acidic groups in the latter either by ester or amide linkage.

925. EMERSON G., BRINK, N. G., HOLLY F. W., KONIUSZY F., HEYL, D., and FOLKERS K.: Vitamin B₁₂. VIII Vitamin B₁₂-like activity of 5,6-dimethylbenzimidazole and tests on related compounds J. Am. Chem. Soc. 72 3084-3085, July 1950.

Two degradation products of vitamin B₁₂ are described which show vitamin B₁₂-like growth activity when fed to rats maintained on a diet devoid of animal protein and containing thyroid powder. These two products are active at milligram-levels in contrast to vitamin B₁₂ which is active at the microgram-level. A third product also has high vitamin B₁₂-like activity while five others have none and one even seemed to depress growth.

Smith Institute for Therapeutic Research, and
Research Laboratories
Smith & Co., Inc.
Baltimore, Md.

926. MAMALIS P., PETROW V., and STURGEON B.: The chemistry of anti-pernicious anaemia factors Part IV Benzimidazole glycosides (1) The preparation and properties of some o-nitroaniline glycosides J. Pharm. and Pharmacol. 2 491-502, Aug. 1950.

Glucosidation of o-nitroaniline gives, in general, a mixture of two isomers designated o-nitroaniline glycosides I and II. In contrast to results reported by Kuhn and Strohle acetylation of these isomers gives the corresponding o-nitroaniline acetylglycosides I and II. Conversion of the Series II isomer into the Series I isomer has been effected in certain cases by very short contact with hot dilute hydrochloric acid. Reduction of the o-nitroaniline acetylglycosides I and II is accompanied in certain instances, by a merging of the two series and the formation of only one o-phenylenediamine acetylglycoside. Similar results have been obtained and are here recorded, employing m-nitro-p-toluidine, 3-nitro-o-4-xyldine and 5-nitro-o-4-xyldine.

927. MAMALIS P., PETROW V., and STURGEON B.: The chemistry of anti-pernicious anaemia factors. Part IV Benzimidazole glycosides. (2) Synthetic routes to the benzimidazole 1-D glucopyranosides J. Pharm. and Pharmacol. 2 503-511, Aug. 1950.

By heating o-phenylenediamine-tetraacetyl-D-glucoside with ethyl orthoformate, 2-ethoxymethylene-o-phenylenediamine tetraacetyl-D-glucoside has been obtained. The foregoing ethyl isocyanide has been converted into benzimidazole-1- β -tetraacetyl-D-glucopyranoside by the action of hot dilute hydrochloric acid. The constitution assigned to this compound has been confirmed by an alternative synthesis from benzimidazole silver and α -acetobromoglucose. Benzimidazole-1- β -D-glucopyranoside has been obtained by hydrolysis of the acetylated glucoside with 6N hydrochloric acid and the pyranoside character of the lactol ring confirmed by periodate titrations. The foregoing novel synthetic methods have been applied to the preparation of 5-methyl-4-5-dimethyl- and 5-6-dimethylbenzimidazole glucosides. In certain cases reaction between an o-phenylenediamine acetylglycoside and ethyl orthoformate has led directly to the formation of the benzimidazole acetylglycoside. The benzimidazole glucopyranosides prepared in the course of this work showed unexpected stability toward hot 6N hydrochloric acid.

928. MAMALIS P., PETROW V., and STURGEON B.: The chemistry of anti-pernicious anaemia factors Part IV Benzimidazole glycosides. (3) The preparation of some benzimidazole pentosides J. Pharm. and Pharmacol. 2 512-518, Aug. 1950.

New methods elaborated in Part IV (2) for the synthesis of benzimidazole glucosides have been extended by the preparation of some benzimidazole pentosides. Results obtained employing the "orthoformate route" show that ethoxymethylene-o-phenylenediamine acetylpentosides undergo more facile ring closure than the corresponding glucosides, being readily converted into the benzimidazole pentosides by the action of alcoholic picric acid. The pyranoside structures assigned to

foregoing compounds have been confirmed by periodate titrations. Benzimidazole pentosides show unexpected stability to acids and are recovered unchanged after heating for 12 hours with 6N hydrochloric acid at 100 C.

- 929 COOLEY G., ELLIS, B., and PETROW V. *The "ninhydrin-reacting" hydrolytic fragment of vitamin B₁₂ and 1-aminopropan-2-ol*, J. Pharm. and Pharmacol. 2 535, Aug. 1950.

In continuation of earlier work the chromatographic behavior of several amino-alcohols of low molecular weight was studied and it was found that 1-amino-propan-2-ol, a structural isomer of 2-amino-propan-1-ol, and ninhydrin reacting fragment are likewise indistinguishable on paper chromatograms irrigated with a number of different solvent systems. Furthermore, oxidation of 1-amino-propan-2-ol with acid potassium permanganate followed by paper chromatography of the product, gives a yellow spot with the ninhydrin reagent, identical in every respect with that obtained from the ninhydrin-reacting fragment in a similar way. Thus, not only is the latter fragment chromatographically inseparable from 1-amino-propan-2-ol, but their respective highly characteristic oxidation products are also inseparable. Independent studies by the Merck group had established the identity of the ninhydrin-reacting fragment with D-1-amino-propan-2-ol by the methods of classical organic chemistry.

930. COOLEY G., ELLIS, B., MAMALIS, P., PETROW V., and STURGEON, B.: *The chemistry of anti-pernicious anaemia factors. Part V. The interrelationship and structure of the α - and γ -components*, J. Pharm. and Pharmacol. 2 579-590, Sept. 1950.

931. BEAVEN G. H., HOLIDAY E. R., JOHNSON E. A., ELLIS, B., and PETROW V. *The mode of linkage of component α in vitamin B₁₂*, J. Pharm. and Pharmacol. 2 733-735, Oct. 1950.

932. BUCHANAN, J. G., JOHNSON A. W., MILLS, J. A., and TODD A. R.: *Chemistry of the vitamin B₁₂ group. Part I. Acid hydrolysis studies. Isolation of a phosphorus-containing degradation product*, J. Chem. Soc. 2845-2855, Oct. 1950.

933. BRINK, N. G., and FOLKERS, K. *Vitamin B₁₂. X. 5,6-Dimethylbenzimidazole a degradation product of vitamin B₁₂*, J. Am. Chem. Soc. 72 4442-4443, Oct. 1950.

Authors' summary: "5,6-Dimethylbenzimidazole has been isolated from an acid hydrolyzate of vitamin B₁₂. Its structure has been established by degradation and by synthesis."

North & Co., Inc.
Rahway, N. J.

934. BOXER, G. E., and RICKARDS, J. C. *Chemical determination of vitamin B₁₂. I. Determination of 5,6-dimethylbenzimidazole by colorimetric and fluorometric methods*, Arch. Biochem. 29: 75-84, Nov. 1950.

A colorimetric and a fluorometric determination of 5,6-dimethylbenzimidazole is described. The colorimetric

method employs the reaction of 4,5-dimethyl-o-phenylenediamine with acetylacetone forming the purple sulfone of 2,4,7,8-tetramethyl-1,5-benzodisulphine. The fluorometric method employs the reaction of 4,5-dimethyl-o-phenylenediamine with alloxan forming the fluorescent 6,7-dimethyl-allyloxazine. These proposed procedures for the determination of benzimidazoles can be used as a basis for an assay of vitamin B₁₂.

North & Co., Inc.
Rahway, N. J.

935. BEAVEN, G. H., HOLIDAY E. R., JOHNSON E. A., ELLIS, B., and PETROW V.: *The chemistry of anti-pernicious anaemia factors. VI. The mode of combination of component α in vitamin B₁₂*, J. Pharm. and Pharmacol. 2 944-955, Dec. 1950.

936. BOXER, G. E., and RICKARDS, J. C. *Chemical determination of vitamin B₁₂. II. The quantitative isolation and colorimetric determination of millimicrogram quantities of cyanide*, Arch. Biochem. 30 572-581, Feb. 1951.

Authors' summary: "One approach to the specific determination of vitamin B₁₂ required a sensitive and specific method for the determination of cyanide. A procedure for the isolation, concentration, and colorimetric determination of 1 part of cyanide in 10¹¹ parts of solution with a precision of 1.5 per cent is described. The factors influencing the isolation of millimicrogram quantities of cyanide by aeration, and the separation of hydrogen cyanide from thiocyanate, hydrogen sulfide, and from other volatile substances interfering with the color reaction, are discussed. The conditions for the color reaction for cyanide described by Epstein have been studied and improvements are proposed which increase the sensitivity and precision of the method."

North & Co., Inc.
Rahway, N. J.

937. BOXER, G. E., and RICKARDS, J. C. *Chemical determination of vitamin B₁₂. III. Methods for the quantitative and specific release of the cyano group of vitamin B₁₂*, Arch. Biochem. 30 582-591, Feb. 1951.

Two procedures for the colorimetric determination of vitamin B₁₂ in microgram quantities are described. Both methods are based on the quantitative liberation and colorimetric determination of cyanide complex bound to the cobalt atom of vitamin B₁₂. In one procedure, the cyano group is released by reduction with hypophosphorous acid. In the other procedure the cyano group is released by exposure to visible light. The determination of cobalamins other than vitamin B₁₂ is described. It is based on the fact that these substances are converted to vitamin B₁₂ by treatment with cyanide. The cyanide thus bound is then determined.

North & Co., Inc.
Rahway, N. J.

938. EMERSON G., HOLLY F. W., SHUNK, C. H., BRINK, N. G., and FOLKERS, K.: *Vitamin B₁₂. XIII. Vitamin B₁₂ like activity of α - and β -ribazole*, J. Am. Chem. Soc. 73 1069-1070, March 1951.

Authors' summary: "A Ribazole (1- α -D-ribofuranosido-5,6-dimethylbenzimidazole) a degradation product of vitamin B₁₂, has been found to elicit a marked vitamin

B₁₂-like response when fed to rats maintained on a diet devoid of animal protein and containing thyroid powder. It is about one four-hundredth as active as vitamin B₁₂ in this test, and on a weight basis may be considered comparable to the activity of riboflavin and pantothenic acid in their respective animal tests. β -Ribazole also gave a significant response in the test.

"1-D-Ribityl-5,6-dimethylbenzimidazole appears to have less vitamin B₁₂-like activity than 5,6-dimethylbenzimidazole."

Wash. Institute for Thoracic Research, and
Research Laboratories
Wash. & Co., Inc.
Bellevue, W. J.

- 939 WRIGHT J. B. *The chemistry of the benzimidazoles*, Chem. Rev. 48 397-541 June 1951.

A review with 769 references.

- 940 DAVIES, M. T., MAMALIS, P., PETROW V., and STURGEON, B. *The chemistry of anti perniciosa anaemia factors. Part VIII. The basicity of some benzimidazoles and benzimidazole glycosides* J. Pharm. and Pharmacol. 3 420-430, July 1951

The pK_a values of 5,6-substituted benzimidazoles in water and/or aqueous ethanol have been determined. The relationship between basicity and constitution in the benzimidazole series is discussed.

- 941 PRIFNER, J. J., DION H. W., and CALKINS D. G. *Further characterization of pseudovitamin B₁₂ pigments* Federation Proc. 11 269 March 1952.

"The harvest broth of a rumen anaerobe has yielded 3 pairs of pigments of the pseudo (ψ) series 3 cyano-cobalt pigments and the corresponding 3 presumably hydrozo compounds. Two pairs (crystalline) are highly active as growth factors for certain lactobacilli but are inactive in chicks, rats and man (pernicious anemia). The 3rd pair (ca. 50% pure) have no demonstrable biological activity but exhibit ultraviolet absorption properties typical of the series. Two crystalline cyano pigments (ψ -vitamins B₁₂ and B₁₂) when subjected to acid degradation have yielded Dg 1-aminopropanol 2, inorganic phosphate, ammonia, a red cobalt fraction, adenine and a trace of hypoxanthine (paper chromatographic evidence). The hypoxanthine is considered to arise from the degradation of adenine. No indication was obtained for the occurrence of 5,6-dimethylbenzimidazole or its derivatives. The crystalline pigments of the ψ -series apparently contain adenine acid in place of 5,6-dimethylbenzimidazole-1- α -D-ribofuranoside-phosphate. The presence of adenine instead of 5,6-dimethylbenzimidazole is in keeping with the higher nitrogen and lower carbon content of the ψ -vitamins as compared to vitamin B₁₂ itself (ψ -vitamin B₁₂ C, 52.06 H, 6.60 N 17.85; Co, 4.40; P 2.31 ψ -Vitamin B₁₂ C, 52.45 H, 6.51; N 18.62 Co, 4.34 P 2.28). Following the cobalamin system of nomenclature for vitamin B₁₂, ψ -vitamin B₁₂ may be referred to as cyano- ψ -cobalamin and ψ -vitamin B₁₂ as cyano- ψ -cobalamin."

Porte, Ruck & Co.
Rivers, Mich.

ANALOGS

942. KACZKA, E., WOLF D. E., and FOLKERS, K.: *Vitamin B₁₂. V Identification of crystalline vitamin B₁₂*, J. Am. Chem. Soc. 71 1514-1515, April 1949 (Letter to Editor)

A new and biologically active crystalline compound, designated vitamin B_{12a}, has been obtained by catalytic reaction of vitamin B₁₂ with hydrogen. It is somewhat less active than vitamin B₁₂. In a single patient with pernicious anemia, Dr. Randolph West, of Columbia University has reported (personal communication) that the parenteral administration of 25 mcg. of vitamin B_{12a} produced about 30 per cent of a maximal hematologic response.

- 943 PIERCE, J. V., PAGE, A. C., JR., STOKSTAD E. L. R., and JUKES, T. H. *Crystallization of vitamin B₁₂*, J. Am. Chem. Soc. 71 2952, Aug. 1949 (Letter to Editor)

A crystalline fraction has been separated from liver extract by chromatography which has absorption spectrum maxima different from those reported for vitamin B₁₂. Two characteristic pink bands were thus separated and were eluted. Fractional precipitation of the first of these with acetone yielded small rodlike red crystals which contained cobalt and phosphorus, and which were biologically active in the chick assay and in the assay with *Lactobacillus leichmannii* 313. The term "vitamin B_{12a}" is suggested for this preparation. The second pink fraction had an absorption spectrum which was characteristic of vitamin B₁₂, and was concentrated to yield needle-like crystals that appeared similar to those of vitamin B₁₂.

- 944 BROCKMAN, J. A., JR., PIERCE, J. V., STOKSTAD E. L. R., BROQUIST H. P., and JUKES T. H.: *Some characteristics of a crystalline compound derived from vitamin B₁₂*, J. Am. Chem. Soc. 72 1042, Feb. 1950 (Letter to Editor)

The isolation of a red crystalline product from vitamin B₁₂ is described. Its biologic activity is similar to that of vitamins B₁₂ and B_{12a}. B_{12a}, which has a band in the absorption spectrum which is missing from the absorption spectra of the new product and vitamin B₁₂, is less effective. The present experimental results indicate that vitamin B_{12a} was produced by hydrogenation of vitamin B₁₂.

Loebke Laboratories
Port River, N. Y.

945. SMITH, E. L. *Recent work on vitamin B₁₂*, Lancet 1: 853, Feb. 25, 1950 (in Soc. Proc.)

Both microbiological assays and partition chromatography have revealed the presence in liver extract of at least three active substances: vitamin B₁₂, an allied vitamin B_{12a}, and thymidine. Also reported was the isolation from *Streptomyces griseus* of another substance which had proved to be as effective clinically as vitamin B₁₂. Its chemical and physical properties were close to those of B₁₂. It is referred to as "the unnamed substance." Investigation of the structure of the substance shows the presence of an atom of cobalt in its molecule, a benzimidazole group, and probably a pyrrolic

molecule of ribose phosphorylated at C₅ or C₆. The cobalt-containing moiety is attached to this phosphorus atom; it represents about two-thirds of the molecule, and its structure has not yet been elucidated. In addition, ammonia and two molecules of Dg L-amino-2-propanol are attached at some point.

The many functions and properties of B₁₂ found by other workers are reviewed.

Glaxo Laboratories Ltd.
Greenford, Middlesex, England

- 959 JACKSON W. G., WHITFIELD G. R., DeVRIES W. H., NELSON H., and EVANS J. S. *The isolation of vitamin B₁₂ from neocyan fermentation*, J. Am. Chem. Soc. 73 337-341 Jan. 1951.

Vitamin B₁₂ has been isolated from liver from aureomycin fermentations and from streptomycin fermentations. The present paper describes an isolation process by which it may be obtained as a co-product of neocyan produced by fermentation. The neocyan fermentation medium is acidified and the vitamin is freed from the mycelium by heating, and adsorbed on carbon. The additional steps by which the isolation is completed and the product purified are described. The final crystalline product occurs as dark red needles.

Crystalline vitamins B₁₂ and B_{12a} are compared by means of three microbiologic assays. The results are in line with the observations of others on the instability of B₁₂ in the presence of certain reducing agents and assay media constituents, which may account for apparent differences in the microbiologic assay of these vitamins. The ultraviolet, visible and infrared absorption curves of the two vitamins are presented.

The Sybille Company
Edinburgh, Scot.

960. PENNINGTON R. J. *A heat-labile vitamin B₁₂ complex in faeces*, Biochem. J. 48 xviii, Jan. 1951 (in Proc. Biochem. Soc.)
961. COOLEY G., ELLIS V., PETROW V., BEAVEN G. H., HOLIDAY E. R., and JOHNSON E. A. *The chemistry of anti-pernicious anaemia factors. Part VII Some transformations of vitamin B₁₂*, J. Pharm. and Pharmacol. 3 271-285, May 1951.

The bathochromic shift observed in the spectrum of vitamin B₁₂ in passing from pH 12 to pH 2 is interpreted as due to aequation of hydroxycobalamin with formation of an aquocobalamin cation. The reaction mechanism underlying the stepwise conversion of vitamin B₁₂ into cyanocobalamin and dicyanocobalamin is discussed. Ammonia cobalochrome, structurally analogous to ammonium haemochrome, has been prepared from vitamin B₁₂ and its conversion into cyanocobalamin effected. Evidence is presented for the existence of histidine cobalochrome, likewise converted into cyanocobalamin by cyanide. A hypothetical scheme for a possible role of vitamin B₁₂ in metabolic processes is presented.

962. BUHS, R. P., NEWSTEAD E. G., and TRENNER, N. R. *An analog of Vitamin B₁₂*, Science 113 625-626, June 1, 1951.

The chemistry of the vitamin B₁₂ cobalt complex is discussed. It is stated that the cyanide ion (CN⁻) may be replaced by other ions or uncharged groups, thus forming analogs of vitamin B₁₂.

In the present experiment the authors have prepared the thiocyanate analog of vitamin B₁₂ by reacting vitamin B₁₂ with potassium thiocyanate. This analog, which crystallizes in purple-red needles, has full biologic activity when compared with vitamins B₁₂ and B_{12a} by several methods of assay. Acute toxicity tests on mice failed to reveal any detectable toxicity at the equivalent level of 3.2 mg. of the analog for a 70-Kg. man, and preliminary clinical reports have indicated that the thiocyanate analog of vitamin B₁₂ is fully active for pernicious anemia.

Chemical and physical properties of the new analog are compared with those of vitamins B₁₂ and B_{12a}. The thiocyanate analog is not compatible with ascorbic acid at a level of 20 mcg./cc. of the analog to 20 mg./cc. of ascorbic acid. Vitamin B_{12a} also reacts with ascorbic acid. Vitamin B₁₂ does not.

Merck & Co., Inc.
Rahway N. J.

- 963 KACZKA, E. A., WOLF D. E., KUEHL, F. A., JR., and FOLKERS, K. *Vitamin B₁₂. XVI Modifications of cyanocobalamin*, J. Am. Chem. Soc. 73 3569-3573, Aug. 1951.

Vitamin B₁₂ (cyano-cobalamin) reacts with sulfuric acid to yield sulfato-cobalamin and with hydrogen sulfide to give a sulfur-containing cobalamin. Vitamin B_{12a} (hydroxo-cobalamin) reacts with chloride, bromide, sulfate, cyanate and nitrite ions to give chloro-, bromo-, sulfato-, cyanato- and nitro-cobalamin. The cobalamin derivatives are converted to vitamin B₁₂ (cyano-cobalamin) by reaction with cyanide ions. An interpretation of the formation of vitamin B₁₂-like compounds from vitamin B₁₂ is discussed.

- 964 COOLEY G., ELLIS V., PETROW V., BEAVEN G. H., HOLIDAY E. R., and JOHNSON E. A. *Some observations on the relationship between vitamins B₁₂ and B_{12a}*, J. Pharm. and Pharmacol. 3 607-608, Sept. 1951.

The contradictory views of various authors on the relationship of vitamins B₁₂ and B_{12a} are reviewed and discussed. No conclusive proof is seen that vitamins B₁₂ and B_{12a} are identical in the solid state.

965. EMERSON G., and FOLKERS K. *Water soluble vitamins. Vitamin B₁₂ or cyanocobalamin and related compounds*, Ann. Rev. Biochem. 20 559-574, 1951.

966. LEWIS, U. J., TAPPAN D. V., and ELVEHJEM, C. A. *A new and biologically different form of vitamin B₁₂*, J. Biol. Chem. 194 539-543, Feb. 1952 (abstr. Blood 7 739 July 1952).

"Since the isolation of vitamin B₁₂ in 1948, numerous forms of the vitamin have been reported. Although B₁₂ and B_{12a} are identical, B_{12b} is a more highly oxygenated form. All of these seem to have the same biologic activity when measured by animal growth responses. This is a

report on characteristics of a material isolated from the feces of rats fed a corn-soybean meal diet supplemented with cobalt sulfate. Although it has growth-promoting properties for *L. leichmannii* and chicks, it is inactive in this respect for the rat. The chromatographic pattern and absorption spectrum of the material are not identical with those of vitamin B₁₂.

University of Warwick
Edgbaston, Birm.

- 957 FORD J. E., HOLDSWORTH, E. S., KOV S. K., and PORTER, J. W. G.: Occurrence of the various vitamin B₁₂ active compounds. *Nature* 171: 150-151, 1953 (abstr. *Blood* 8: 486-487 May 1953)

"Many of the substances with activity similar to that of vitamin B₁₂ are probably not pure substances. In this paper are discussed the possible causes of activity of

pseudo-vitamins B₁₂ and B₁₂, factor A, vitamin B₁₂, B₁₂, factor WII, factor B and factor C.

"The vitamin B₁₂ activity for micro-organisms of extracts of gut contents and feces is contributed in varying proportions by five substances, viz. factors A, B, and C, pseudo-vitamin B₁₂, and vitamin B₁₂ itself. In natural materials these compounds may exist in both free and combined forms.

"As these vitamin B₁₂ active compounds are formed by microbial synthesis in the rumen or large intestine or both, it is probable that the relative amounts of each that appear in gut contents depend on the composition of the microbial flora. This will vary with the nature of the diet."

University of Reading
Reading, England

U S PHARMACOPEIA U S PATENT OFFICE

958. U. S. P. REVISION COMMITTEE: *Vitamin B₁₂ ad*mitted to U. S. P. XIII J. Am. Pharm. A. (Pract. Pharm. Ed.) 10: 544, Sept. 1949

Crystalline vitamin B₁₂, crystalline vitamin B₁₂ injection, and vitamin B₁₂ concentrate are being admitted to the U. S. P. XIII by Interim Revision. Recommendations on claims for vitamin B₁₂ are quoted.

- 959 UNITED STATES PHARMACOPEIA: U.S.P. Committee by interim revision admits vitamin B₁₂ to U.S.P. XIII Merck Rep. 59: 7 Jan. 1950.

Because of the immediate importance of vitamin B₁₂ in the treatment of pernicious anemia, the U.S.P. Revision Committee is admitting to U.S.P. XIII by interim revision, rather than waiting for publication of U.S.P. XIV the following items: Crystalline Vitamin B₁₂, Crystalline Vitamin B₁₂ Injection, Vitamin B₁₂ Concentrate. Since a reliable microbiological assay for the vitamin B₁₂ content of commercial anti-anemia preparations has not yet been standardized, U.S.P. units are to be used for the present.

970. COUNCIL ON PHARMACY AND CHEMISTRY: *Vitamin B₁₂-U.S.P.—Cobione (Merck)* J.A.M.A. 143: 1342-1343, Aug. 12, 1950.

Cobione has been accepted by the Council. Crystalline vitamin B₁₂ has hemopoietic activity apparently identical with that of the antianemia factor of liver. It has, however, not been established as the complete or essential counterpart of this factor. Studies indicate that vitamin B₁₂ is clinically efficacious in the treatment of pernicious anemia, with or without neurologic complications, tropical and nontropical sprue, nutritional macrocytic anemia due to vitamin B₁₂ deficiency and in certain cases of megaloblastic anemia of infancy. Cobione is particularly useful for treating patients who are sensitive to liver extract. The Council is not as yet able to make a statement as to the effectiveness of crystalline vitamin B₁₂ as compared with liver extract in the treatment of spinal cord lesions associated with pernicious anemia, because the follow-up period on such patients has not been sufficiently long. The

effects of crystalline vitamin B₁₂, thus far observed, however have been as good as those of liver extract.

In man, no toxic reactions due to Cobione have been reported; the drug is also not toxic to animals when given orally or subcutaneously. The minimum effective dosage of crystalline vitamin B₁₂ is believed to be about 1 mcg. a day usually given in multiples of this amount at longer intervals, for instance 15 mcg. every two weeks. One microgram of Cobione is estimated to be about equal biologically to one U.S.P. "injectable" unit of liver extract. Recommended parenteral Cobione dosages are as follows: In uncomplicated pernicious anemia, 15 mcg. once or twice a week until remission occurs, then a maintenance dose of 15 mcg. every other week. In pernicious anemia with neurologic complications, 15 to 30 mcg. once or twice weekly followed by a maintenance dose of 15 mcg. every other week. In sprue, 15 to 30 mcg. once or twice weekly until remission occurs, followed by 15 mcg. once a week to prevent relapse. In megaloblastic anemia of infancy the dosage is the same as for sprue, but if remission is not prompt, folic acid should be given instead of vitamin B₁₂. In nutritional macrocytic anemia, a single dose of 15 mcg. of Cobione will usually produce a favorable initial response, but this dose must sometimes be repeated at two-week intervals to prevent relapse. Satisfactory responses are obtained with oral Cobione, when high doses are given. Most patients respond slowly to oral doses of vitamin B₁₂ which are by weight 50 to 60 times the size of those used in parenteral therapy. This amount can be reduced by giving the vitamin along with normal gastric juice.

- 971 WOLF F. J.: *Process for recovering vitamin B₁₂*, U. S. 2,530,416 Official Gaz. U.S. Pat. Off. 640: 779 Nov. 21, 1950.

"In a process for recovering vitamin B₁₂ from a starting material selected from the class consisting of fermentation broths obtained by the propagation of a vitamin B₁₂ producing microorganism in a suitable nutrient medium and concentrates thereof, the step that comprises treating the starting material with a substance furnishing cyanide ions, thereby obtaining a mixture containing an increased amount of vita

972. RICKES, E. L., and WOOD T. R. *Vitamin B₁₂*. U. S. 2,563,794 Official Gaz. U. S. Pat. Off. 649 302, Aug. 7 1951.

"The compound vitamin B₁₂, an organic substance containing cobalt, together with carbon, nitrogen, hydrogen, oxygen and phosphorus, said compound being a red crystalline substance soluble in water methyl and ethyl alcohol and phenol, and insoluble in acetone, ether and chloroform, and exhibiting strong absorption maxima at about 2780Å, 3610Å and 3500Å, and an L.D. activity of about 11,000,000 L.D. units per milligram."

973. THE CHEMICAL WORLD THIS WEEK: Method for producing vitamin B₁₂. Chem. & Engin. News 30 1746, April 28, 1952.

U.S.D.A. patent 2,561,364. "Vitamin B₁₂ is produced by cultivating *Flavobacterium devorans* in a medium containing assimilable carbon source and an assimilable nitrogen source. The product is recovered as

a vitamin concentrate by evaporating the culture liquor after fermentation is complete."

974. PATTERSON A. M.: *Amino acids carotenoids, vitamins*, Chem. & Engin. News 30 104, Jan. 7 1951.

The following names have been adopted by the IUPAC Commission of the Nomenclature of Biological Chemistry:

B ₁ (aneurin, thiamine)	thiamine
B ₂ (riboflavin)	riboflavin
B ₁₂ (collectively)	cobalamin
B ₁₂ (pure)	cyanocobalamin
B _{12a}	hydroxycobalamin
B _{12b}	nitrocobalamin
E	a.s. and γ-tocopherol
PP (niacinamide, nicotinamide)	nicotinamide

The names p-aminobenzoic acid, ascorbic acid, biotin, choline, and pantothenic acid are confirmed.

MICROBIOLOGIC ASSAYS

975. SHORR, M. S. *Activity of vitamin B₁₂ for the growth of Lactobacillus lactis*, Science 107 397 898, April 16, 1948.

The minute amount of the new crystalline compound isolated from liver vitamin B₁₂, which is required by *Lactobacillus lactis* Dorner for growth places it among the most potent microbiologically active compounds. Its extremely high potency, 11,000,000 units per mg. with a 23-hour growth period and 17,000,000 units per mg. with one of 42 hours, compared with an assigned potency of 1,000 units per mg. for the arbitrary liver concentrate standard used in the LLD factor assays, suggests that it is wholly or partially responsible for the LLD growth activity observed with liver extracts. The above standard was free from the other factor T₃ also required by *L. lactis*, at levels as high as 500 micrograms per tube. Vitamin B₁₂ has little or no effect on the growth of *L. casei*, *L. fermenti*, *L. arabinosus*, *Streptococcus faecalis* R, and *Escherichia coli*. These organisms also grow readily without supplementation with T₃ but they have been found in synthesizing this factor.

Studies of source materials reported to contain unidentified factors for chicken nutrition revealed that LLD factor activity occurs in fairly high amounts, in approximately decreasing order in a papain digest of acid precipitate from cow manure, the acid precipitate from cow manure, fish meal, pancreatin, papain, egg white, and egg yolk, and in lower amounts in alcoholic extract of whey potassium permanganate-oxidized alcoholic extract of whey soybean oil meal, gelatin, zein, and Mylase P enzyme. T₃ factor activity is also found, in approximately decreasing order in the papain digest of acid precipitate from cow manure, the acid precipitate of cow manure, egg yolk, papain, and pancreatin, but in much lower amounts in fish meal, alcoholic extract of whey soybean oil meal, crude casein, egg white, zein, Mylase P enzyme, potassium permanganate-oxidized alcoholic extract of whey and gelatin. The distribution of LLD and T₃ factor activities in these materials suggests that they may be involved in chicken nutrition.

976. SHORR, M. S., and BRIGGS, G. M. *The effect of dissociation in Lactobacillus lactis cultures on the requirements for vitamin B₁₂*, J. Biol. Chem. 176 1463-1464, Dec. 1948 (Letter to Editor).

Although *Lactobacillus lactis* Dorner is stable in its requirement for both vitamin B₁₂ (LLD factor) and the tomato juice (T₃) factor when cultured on a tomato-juice, yeast-extract, skim-milk medium, the culture undergoes dissociation when serial transfers are made. The medium used in the assay of the LLD factor and in connection with the isolation of vitamin B₁₂ is that of Baumgarten et al. (J. Am. Chem. Soc. 66 1607 1944) modified by the addition of all the B vitamins at tenfold concentration, except biotin and pteroylglutamic acid, which are added at 0.05 microgram per cc. Adenine, guanine, uracil, and xanthine are also added, together with 0.5 cc. of T₃ factor. This assay works well with the stabilized culture and purified liver extracts or crystalline vitamin B₁₂ but erratic results may be obtained with the dissociating culture or with some crude materials because of inhibitory substances. This inhibition is partly due to high concentrations of folic acid. High levels of serine, para-aminobenzoic acid, xanthine, and certain salts also inhibit growth. Culture filtrates of *Lactobacillus casei*, *Streptococcus faecalis* R, and *Escherichia coli* and fresh chicken droppings have low vitamin B₁₂ activity.

977. HOFFMANN C. E., STOKSTAD E. L. R., FRANKLIN, A. L., and JUKES, T. H.: *Response of Lactobacillus leichmannii 313 to the anti-pernicious anemia factor* J. Biol. Chem. 176 1465-1466, Dec. 1948 (Letter to Editor).

Thymidine has a growth-stimulating effect upon *Lactobacillus lactis* Dorner under conditions in which the growth is also promoted by vitamin B₁₂. Thymidine also promotes the growth of *L. leichmannii* 313. The effect of a sample of crystalline anti-pernicious anemia (APA) factor was tested with this organism. The results indicate that *L. leichmannii* 313 may be a sensitive test organism for the APA factor and that liver extract produces a similar response.

978. SKEGGS, H. R., HUFF, J. W., WRIGHT, L. D., and BOSSHARDT, D. K.: *The use of Lactobacillus leichmannii in the microbiological assay of the "animal protein factor"* J. Biol. Chem. 176 1459-1460, Dec. 1948 (Letter to Editor)

Lactobacillus leichmannii has given very satisfactory results as an assay organism for the "animal protein factor." Recent reports from the Merck laboratories have indicated that the "animal protein factor" is identical with or closely related to vitamin B₁₂. If this is true, it is probable that *L. leichmannii* may be the organism of choice for the microbiologic assay of vitamin B₁₂. *Lactobacillus lactis* is currently used for the assay of vitamin B₁₂, but this organism has very complicated growth requirements, which makes its use difficult. Both *L. leichmannii* and *L. lactis* respond to purified liver preparations (known to be rich in "animal protein factor") and both organisms are stimulated in growth by thymidine.

979. HUTNER, S. H., PROVASOLI, L., STOKSTAD, E. L. R., HOFFMANN, C. E., BELT, M., FRANK, L. N. A., and JUKES, T. H.: *Assay of anti-pernicious anemia factor with Euglena*, Proc. Soc. Exper. Biol. & Med. 70 118-120, Jan. 1949

The algal flagellate *Euglena gracilis* var. *becillaris* exhibits a quantitative growth response to crystalline anti-pernicious anemia factor (vitamin B₁₂) when grown in a basal purified culture medium. Thymidine is inactive, although it is effective for *Lactobacillus*. This suggests that biochemical generalizations regarding the functions of anti-pernicious anemia factor should not be made solely on the basis of observations with *Lactobacillus*.

980. SHAW, G. E.: *Liver extracts*, Lancet 1 239-240 Feb. 5, 1949 (In Letters to the Editor)

The writer thinks it probable that some modification of the *Lactobacillus lactis* Dorner assay of vitamin B₁₂ will eventually provide a unitage system for describing the anti-pernicious anemia potency of liver extracts. A technique for this assay has been evolved in the Evans Biological Institute, and an alternative cup method for the determination of LDO growth factors has recently been announced by Cathbertson. If a liver extract contained only clinically active vitamin B₁₂, the total LDO activity would measure the clinical potency. Some growth factors for *L. lactis*, however, have no clinical activity against anemia. It is suggested that by means of paper chromatography the amount of each factor present in a particular extract might be determined. If a number of liver extracts with different spectra were assayed clinically it would probably be possible to translate vitamin B₁₂ microbiologic activity into clinical potency. Such experiments are under way.

981. SMITH, E. L., and CUTHBERTSON, W. F. J.: *Liver extracts*, Lancet 1 825, Feb. 19 1949

Microbiological assay has been used to supplement, though in no way to replace, clinical tests. It has been found that liver extracts contain four substances active in the microbiological assay of which only two have been proved clinically active.

It is hoped that clinical tests may eventually be replaced by combination of chromatography (paper strip?) and microbiological assay but comparison of the two methods on a large number of batches can alone provide information to justify such a procedure.

982. CAPPS, H. F., HOBBS, N. L., and FOX, S. H.: *A method for the microbiological assay of vitamin B₁₂*, J. Biol. Chem. 178 517-518, March 1949 (Letter to Editor)

In the microbiologic assay of vitamin B₁₂, *Lactobacillus leichmannii* may be preferable to *Lactobacillus lactis* Dorner as the test organism, since it shows less tendency to dissociate. The standard (purified vitamin B₁₂, Merck) and test materials are diluted to 0.02 mcg. per cc. in distilled water. The test organism responds best within the range of 0.01 to 0.10 mcg. per tube levels. Duplicate and triplicate tubes on each level give growth response in good agreement turbidimetrically. Tubes containing only distilled water and the basal medium without B₁₂ produce no growth. Further studies are under way to determine the relative merits of this method of assay of natural materials containing minute amounts of vitamin B₁₂ and a similar method in which *Lactobacillus lactis* Dorner is used as the test organism.

983. STOKSTAD, E. L. R., DORNBUCH, A. C., FRANKLIN, A. L., HOFFMANN, C. E., HUTCHINGS, B. L., and JUKES, T. H.: *Microbiological assay of vitamin B₁₂ by Lactobacillus leichmannii* Federation Proc. 8 257 March 1949

Assay of vitamin B₁₂ by means of the growth response of *Lactobacillus leichmannii* is discussed, particularly in regard to the action of thiolglycolic acid and other substances in protecting the vitamin from destruction during autoclaving of the samples with the medium.

984. GREENE, R. D., BROOKS, A. J., and MCCORMACK, R. B.: *Some conditions which affect the assay of vitamin B₁₂ with Lactobacillus lactis Dorner* J. Biol. Chem. 178 999-1000, April 1949

The assay of vitamin B₁₂ with *Lactobacillus lactis* Dorner is influenced by the degree of aerobiosis in the broth cultures. Three minutes autoclaving in 23 x 150 mm. tubes gave consistent assays. The growth promoting activity of B₁₂ for *L. lactis* and *L. leichmannii* was completely inhibited by folic acid and almost completely by aminopterin, while teropterin and N¹⁰ methylpterols acid were not antagonistic.

985. SHAW, G. E.: *Lactobacillus lactis Dorner for the assay of vitamin B₁₂*, Nature 164 186-187 July 30, 1949 (Letter to Editor)

A technique is described in detail for obtaining consistent results in *Lactobacillus lactis* Dorner assays of vitamin B₁₂, which previously have shown wide variations. This organism shows a remarkable proneness to change its morphology under different cultural conditions, and these changes alter its growth characteristics and sensitivity to vitamin B₁₂. A uniform degree of insensitivity is difficult to insure, but in the Evans Biological Institute the organism has been maintained in a co

condition of high sensitivity from a single colony isolation through more than 150 daily subcultures on agar containing 1% "Evans Bacteriological Peptone," 0.1% "Evans Hepamine," 0.1% "Tween 80," and either 5% tomato juice or 0.01% potato extract fraction.

986. KODITSCHKE, L. K., HENDLIN D., and WOODRUFF H. B. *Investigations on the nutrition of Lactobacillus lactis Dornier* J Biol. Chem. 179 1093-1102, July 1949

An unidentified growth factor LLD, which is required by *Lactobacillus lactis Dornier* has been reported by Shorb. Efforts to utilize *L. lactis Dornier* for assay of the LLD growth factor have shown that the requirement is affected by various environmental factors, which are described.

987. CASWELL, M. C., KODITSCHKE, L. K., and HENDLIN D.: *The microbiological estimation of Lactobacillus lactis Dornier activity with vitamin B₁₂ as a standard*, J Biol. Chem. 180 125-131 Aug 1949

The authors describe in detail the method of assay for vitamin B₁₂ used in the Merck laboratories, where this vitamin was first isolated. It is based upon the response of *Lactobacillus lactis Dornier* variant 6a to vitamin B₁₂, as measured by the lactic acid formed from the decarboxylation of glucose. The specific effect of oxygen tension on the assay and means of eliminating such effect are discussed. In an addendum, the authors state that a threefold increase in precision has been obtained in the assay by substituting *L. lactis Dornier* ATCC 8000 for the 6a variant. A precision range of ± 20 per cent can be reached with only six replicates.

988. SHAW G. E.: *Notes on microbiological assays using Lactobacillus lactis Dornier* J Pharm. and Pharmacol. 1 695-700 Oct. 1949

A basal medium (consisting of H₂SO₄, hydrolyzed casein, tryptophane, vitamin B complex, Tween 80 and specially prepared T₃ Factor) and a procedure for the microbiological assay of vitamin B₁₂ are described, which have given reproducible results over a long period.

989. PEELER, H. T., YACOWITZ, H., and NORRIS, L. C.: *A microbiological assay for vitamin B₁₂ using Lactobacillus leichmannii*, Proc. Soc. Exper Biol. & Med. 71 515-521 Nov 1 1949

A microbiologic assay for vitamin B₁₂ using *Lactobacillus leichmannii* has been developed. The assay medium contains crystalline amino acids and the nitrogen source and adsorbed tomato juice filtrate as a source of unidentified factors. A high level of cysteine and ferrous sulfate in the medium keeps it in the necessary reduced state. This method appears to be specific for vitamin B₁₂. A tabulation of the results of its application to various food substances showed the highest content of vitamin B₁₂ in Wilson's liver I, (399 mcg./Gm.) red fish meal (111 mcg./Gm.) and crude casein (104 mcg./Gm.) and the lowest in wheat (7 mcg./Gm.) and dried brewers yeast (8 mcg./Gm.)

Received October 11, 1949
Published, Nov. 7

990. FOSTER, J. C., LALLY J. A., and WOODRUFF H. B.: *Cup assay with vitamin B₁₂ as a standard*, Science 110 507-509 Nov 11, 1949

The microbiologic estimation of LLD (*Lactobacillus lactis Dornier*) activity by means of *Lactobacillus lactis* or *Lactobacillus leichmannii*, using vitamin B₁₂ as a standard, is influenced by degree of aeration, by oxidation-reduction potential, and by accumulation of peroxides. These factors are difficult to control in the titrimetric assay procedure. The cup assay method proposed by Heasley for the estimation of penicillin, which has been used in assays for biotin, thiamine, and riboflavin, allows more rigid control of influential factors and hence gives much more precise results. Also, it is possible by this method, by use of abnormal salt concentrations, to eliminate the diffuse growth response of a test culture to deoxyribonucleic acid or its constituent nucleosides, whereas the Shorb titrimetric assay shows LLD activity for these substances. Cup and titrimetric assays show somewhat different responses to modified substances, such as vitamin B₁₂, when assayed against a vitamin B₁₂ standard. Impure materials, containing various LLD active substances, do not necessarily yield equal results by both methods. Shorb's liver concentrate standard, assigned a value of 1,000 LLD units per mg. by titrimetric assay contained 1,120 units per mg. by cup assay with crystalline vitamin B₁₂ as a standard.

Received Oct. 11, 1949
Published Nov. 7

991. HOFFMANN C. E., STOKSTAD E. L. R., HUTCHINGS B. L., DORNBUSH, A. C., and JUKES, T. H.: *The microbiological assay of vitamin B₁₂ with Lactobacillus leichmannii*, J Biol. Chem. 181 635-644, Dec. 1949

An assay method for B₁₂ using one of two strains of *Lactobacillus leichmannii* is described. One strain grows more rapidly than the other thus shortening the assay period, but both give satisfactory results. To prevent destruction of B₁₂ when the assay tubes are autoclaved it is necessary to add a reducing agent. Alternatively, sterile assay samples can be added to the autoclaved medium. A growth factor was produced in the medium during autoclaving unless sucrose was substituted for glucose. The growth factor was not needed if thioglycolic acid or an extract of asparagus was included in the medium. The effect of deoxyribonucleosides, which replace vitamin B₁₂ for the growth of *L. leichmannii*, was measured by assaying the samples before and after alkaline hydrolysis which destroyed vitamin B₁₂.

992. WHITE, W. F., MOTE, J. R., and HAYS E. E.: *A preliminary study of the relationship between Lactobacillus lactis Dornier response and clinical antiperidone anemia activity of liver extracts*, Blood J Hematol. 4 1357-1360 Dec. 1949

Received October 11, 1949
Published, Nov.

993. KOCHER, V.: *Zur Technik der mikrobiologischen Bestimmung von Vitamin B₁₂ mit Lactobacillus lactis* (The microbiologic determination of vitamin B₁₂ with *Lactobacillus lactis*) Intern. Z. Vitaminforsch. 20 369-374, 1949

In a synthetic medium containing tomato juice and "peptone witte" (1) *L. lactis* 1175 requires vitamin B₁₂ for growth under aerobic, but not under anaerobic, conditions. Without the addition of 1 B₁₂ is required both under anaerobic and aerobic conditions. Vitamin C may substitute for B₁₂ in both cases.

- 994 SHAW G. E. Vitamin B₁₂ with some notes on *Lactobacillus lactis* Dorner. *Australasian J Pharm* 30 97 1949

A review of the growth factor vitamin B₁₂.

- 995 CUTHBERTSON W. F. J. Estimation of the anti-pernicious anaemia factor. *Biochem. J* 44 No. 2 v 1949 (In Soc. Proc.)

The author has been unable to obtain a response to anti-pernicious anaemia factor (APAF) using the method of Shorr and Briggs for B₁₂ assay (*J Biol. Chem.* 176 1463, 1948 [Abstr. 976]) since the medium used by these investigators appears to be deficient in growth factors for the strain of *Lactobacillus lactis* with which he has been working. He describes a cup plate assay method which is adaptable to the determination of APAF activity and to the detection of other members of the B₁₂ group of microbiologic growth factors in purified liver extracts. The medium employed is essentially that of Roberts and Snell (*J Biol. Chem.* 163 499 1945) with the addition of tomato juice, Tween 80, and 2 per cent agar. Under the conditions of test, the diameters of the zones of microbiologic activity are proportional to the logarithms of the APAF concentrations over the range of 0.02 to 0.05 γ of crystalline APAF per cc. Both of these factors contribute to microbiologic activity and unless the ratio of clinical to microbiologic activity is the same for both, this test alone will not predict exactly the clinical potency of liver extracts. Although the method is relatively insensitive and somewhat susceptible to interference by other members of the B₁₂ group, preservatives, and antibiotics, this is usually easy to detect and the procedure has the advantage of speed and simplicity.

Chem. Laboratories Ltd.
Camford, Wiltshire, England

- 996 THOMPSON H. T., DIETRICH, L. S., and ELVEHJEM, C. A. The use of *Lactobacillus leichmannii* in the estimation of vitamin B₁₂ activity. *J Biol. Chem.* 184 175-180, May 1950.

Authors summary: "A microbiological assay for vitamin B₁₂ activity for *Lactobacillus leichmannii* ATCC 4797 has been developed. Satisfactory standard curves, low blanks, and satisfactory recovery values were obtained.

"A method for the release of vitamin B₁₂ activity from animal material has been described.

"The vitamin B₁₂ activity of various animal materials has been presented."

University of Minnesota
Madison, Wis.

- 997 SKEGGS, H. R., NEPPLE, H. M., VALENTIK, K. A., HUFF, J. W., and WRIGHT, L. D.: Observations on the use of *Lactobacillus leichmannii* 4797 in the microbiological assay of vitamin B₁₂. *J Biol. Chem.* 184 211-221 May 1950.

An assay procedure for vitamin B₁₂ employing *Lactobacillus leichmannii* 4797 is described. This procedure has been found reliable and reproducible. Various problems encountered in the use of this method are discussed.

Shaw and Bohmer Inc.
Chesham, Pa.

- 998 BESSEL, C. J., HARRISON E., and LEES K. A. Assay of vitamin B₁₂ with a mutant of *Escherichia coli*. *Chem. & Ind.*, p. 561 July 15, 1950.

Using the cup-plate procedure previously described for the assay of auroin and riboflavin, and also of vitamin B₁₂ with *Lactobacillus lactis* Dorner, an assay method for B₁₂ with a mutant of *E. coli*, requiring B₁₂ for growth, has been developed. The method is less sensitive than the tube method, involving the use of *Lactobacillus leichmannii* 318, but more sensitive than the plate methods with either *L. leichmannii* or *L. lactis* Dorner.

- 999 LOY H. W., JR., HAGGERTY J. F., and KLINE, O. L. A cause of variation in the microbiological assay for vitamin B₁₂. *Arch. Biochem.* 29 451, Dec. 1950 (In Letters to Editor)

The importance of adequate rinsing of glassware used in microbiological assays for vitamin B₁₂ is discussed. The presence of small amounts of detergents on glassware causes inhibition of the growth of the test organisms, giving incorrect B₁₂ values.

Food and Drug Administration
Washington 25, D. C.

- 1000 HENDLIN D., and SOARS, M. H. Comparative microbiological studies with vitamin B₁₂ and B₁₂ase. *J Biol. Chem.* 188 603-610 Feb. 1951.

Authors summary: "The microbiological activities of vitamin B₁₂ and B₁₂ase depend markedly upon the assay organism and technique employed.

"In the *Lactobacillus leichmannii* assay the growth-promoting effect of vitamin B₁₂ is destroyed by autoclaving with the medium; vitamin B₁₂ is unaffected. This destruction is prevented by the addition of suitable quantities of thioglycolic or ascorbic acid. Since many liver samples show a comparable loss of activity upon autoclaving with the *L. leichmannii* assay medium, it appears that they contain appreciable quantities of vitamin B₁₂ or B₁₂ase-like substances.

"In the case of *Lactobacillus lactis* there appears to be no loss of microbiological activity of vitamin B₁₂ or B₁₂ase during autoclaving with the assay medium. The activity of both vitamins was increased by autoclaving and the addition of reducing substances. In addition these conditions lead to an increase in the potency of vitamin B₁₂ and so it reaches the biological activity of vitamin B₁₂ase.

"No apparent differences in microbiological activity were observed between vitamin B₁₂ and a substance described in the literature as vitamin B₁₂ase."

Novak & Co., Inc.
Babson, N. J.

- 1001 LAIKIN F. E., and STUCKEY R. E.: Some observations on the cup-plate assay of vitamin B₁₂ using *Lactobacillus lactis* Dorner 10697. *J Biol. Chem.* 184 154-154 March 1951

The cup-plate assay with *L. lactis* Dorner 10697 is more reliable than tube tests with various organisms. The effect of the pH and cresol concentrations was studied. The assay was found to be particularly useful in determining the stability of samples of vitamin B₁₂ of differing purities. With other vitamin B₁₂ preparations the microbiological assay was always interpreted in conjunction with organic cobalt determinations. Assays of vitamin B₁₂ concentrates obtained from *Streptomyces fermentans* liquors gave results that were difficult to interpret, and it was considered necessary to use the assay in conjunction with a chromatographic procedure, although some difficulty was experienced in getting a quantitative estimate of the small amounts present on the chromatogram.

1002. CUTHBERTSON, W F J., PEGLER, H. F., and LLOYD J T. *The assay of vitamin B₁₂. Part III Microbiological estimation with Lactobacillus lactis* Dorner by the plate method, *Analyt 76* 133-140 March 1951.

The cup plate method for the rapid microbiological assay of sucrose and riboflavin has been found applicable to the estimation of vitamin B₁₂ with a suitable strain of *Lactobacillus lactis* Dorner. Factors affecting the "zone" response of this organism to vitamin B₁₂ have been investigated; they include the effects of vitamins B₁₂ and the decarboxides, times of incubation and standing before incubation, range of concentrations and standing before incubation, range of concentrations of the vitamin and density of the inoculum. The standardized assay permits rapid estimates of potency over a range sufficiently wide and with fiducial limits sufficiently narrow for routine purposes, without the use of an inconveniently large number of assay plates.

- 1003 EMERY W B, LEES, K. A., and TOOTILL, J P R.: *The assay of vitamin B₁₂. Part IV The microbiological estimation with Lactobacillus leichmannii* 313 by the turbidimetric procedure *Analyt 76* 141-146, March 1951.

Details are presented of a microbiological tube assay for vitamin B₁₂ with *Lactobacillus leichmannii* 313 as test organism. Statistical analyses of a (8 + 3) assay and a standard response curve are given and show that the method is sufficiently sensitive and accurate for routine use.

- 1004 BROQUIST H. P., STOKSTAD, E. L. R., and JUKES, T. H.: *Further observations on the microbiological assay for vitamin B₁₂ using Lactobacillus leichmannii*, *Proc. Soc. Exper. Biol. & Med.* 76 806-811, April 1951.

In an attempt to compare vitamin B₁₂ with vitamin B₁₂, the technique of assaying the two vitamins by aseptic addition to the medium was reinvestigated. It was repeatedly found that the growth response of *Lactobacillus leichmannii* was less when vitamin B₁₂ was added aseptically to the medium than when this vitamin was autoclaved in the medium. In contrast, liver extract, vitamin B₁₂, B₁₂, and amino-cobalamin (Compound AC2, Drug Houses) gave essentially the same growth when added to the culture medium aseptically or autoclaved. As a consequence, erroneously high

were obtained when these samples were assayed against vitamin B₁₂ using aseptic technique.

By means of paper strip chromatography it was demonstrated that when vitamin B₁₂ is autoclaved with thiomalic acid, the vitamin is transformed into a new compound which is distinct from vitamin B₁₂ and is more potent microbiologically when the compounds are compared by aseptic assay technique.

The significance of these results in the interpretation of the microbiologic assay of the vitamin B₁₂ content of natural materials when vitamin B₁₂ is used as a standard is briefly discussed.

An addendum states that during the progress of this work the authors received a copy of a paper by H. W. Long and O. L. Kluge (in press) in which it was postulated that both vitamin B₁₂ and vitamin B₁₂ are converted to a form of vitamin B₁₂ more readily utilisable by *L. leichmannii* after heating in the basal medium.

Lactobacillus leichmannii
Food Research, N. Y.

1005. CUTHBERTSON W F J., and LLOYD J T. *The assay of vitamin B₁₂. I Factors affecting the response of Lactobacillus lactis* Dorner ATCC 8000 to vitamin B₁₂, *J. Gen. Microb.* 5 416-420, May 1951.

1006. SOARS, M. H., and HENDLIN, D. *The use of potassium cyanide in the Lactobacillus leichmannii assay for vitamin B₁₂*, *J. Bact.* 62 15-17 July 1951.

- 1007 COOPERMAN J M., DRUCKER, R., and TABENKIN B. *Microbiological assays for vitamin B₁₂. A cyanide enhancement effect* *J. Biol. Chem.* 191 135-141 July 1951.

An improved assay for vitamin B₁₂ utilizing *Lactobacillus lactis* Dorner ATCC 8000, has been described which incorporates KCN in the basal medium. Results comparable to those obtained with *Lactobacillus leichmannii* have been obtained on a variety of natural and partially purified vitamin B₁₂-containing materials.

1008. BROWNLEE, K. A., and LAPEDES D N.: *The effect of design upon the error of a microbiological assay for vitamin B₁₂*, *J. Bact.* 62 433-444, Oct. 1951.

Statistical Concepts for Medical Research
New Brunswick, N. J.

1009. BURKHOLDER, P R.: *Determination of vitamin B₁₂ with a mutant strain of Escherichia coli*, *Sci. Cites* 114 459-460, Nov. 2, 1951.

1010. " " ISON E., LEES, K. A., and WOOD, F.: *Assay of vitamin B₁₂. Part VI Microbiological assay with a mutant of Escherichia coli by the cup assay* *Analyt 76* 696-705, Dec. 1951.

Cup assay of vitamin B₁₂
mutant of *Escherichia coli*
Lab. Invest. 3: 215-216, 1952.

1012. TARR, H. L. A.: *Microbiological assay of vitamin B₁₂ in crude materials*, Federation Proc. 11 297 298, March 1952.

"Work in two different laboratories has shown that addition of cyanide but no reducing agent to media in titrimetric assays has given similar results with cyano- and hydroxo-cobalamins. Titrimetric procedures are slow and use of cyanide without reducing agent in Skeggs (1950) medium resulted in slow growth, while simultaneous use of reducing agent and cyanide permitted rapid growth but rarely gave the same results with the different cobalamins. Aseptic addition of both cyano- and hydroxo-cobalamins to the medium using 0.5 mg./ml. of mercaptosuccinic acid gave identical turbidimetric assay curves, while use of ascorbic acid under such conditions had a pronounced destructive action on hydroxo-cobalamins. Sterilization in the medium normally enhanced growth with cyano-cobalamins but had a marked destructive effect on hydroxo-cobalamins. These findings are important because in crude protein materials much of the vitamin B₁₂ occurs as hydroxocobalamins or similar form chromatographically (Woodruff and Foster KH₂PO₄-paper method). When 500 mg. of cyano- or hydroxo-cobalamins was added to 1-gm. samples of fish meal and these were autoclaved 5 minutes at 120 C., 95-110% of the added vitamin could be recovered by aseptic addition turbidimetric assay. Chromatographic separation of cyano- and hydroxo-cobalamins from such preparations (2.5 mg.) followed by elution and combined assay gave 86-89% recovery. With this method of assay heating crude materials with a reducing agent such as mercaptosuccinic acid may cause fictitiously high recovery due to formation of a more reactive form of the vitamin. Proteolysis with enzymes often occasions formation of high concentrations of uncharacterized vitamin B₁₂-active substances."

Public Health Experimental Station,
Fancouver, B. C., Canada

- 1013 WILLIAMS W. L., ESPOSITO R. G., PIERCE, J. V. *A rapid simple assay method for vitamin B₁₂ and other* Federation Proc. 11 458, March 1952.

"The vitamin B₁₂-requiring *E. coli* mutant, 113-3 (Davis and Mingioli) *J. Bact.* 60: 17 1950 [1034]) has been utilized for the assay of vitamin B₁₂ means of a new rapid simplified technique. Test are on the basal medium solidified with agar. Zones growth are measured at any time from 12 hours to 3 The basal medium is simple and chemically defined consisting of inorganic salts and glucose. Pads known amounts of vitamin B₁₂ can be prepared in advance so that standard curves can be prepared in a few minutes. Aseptic conditions are not essential. One or less time is required to carry out the pad-plate procedure compared to the test-tube technique. The has the fundamental advantage that vitamin B₁₂ vitamin B₁₂, as well as amino-cobalamins, nitro-cobalamins and sulfite-treated cyano-cobalamins all have activity. Relatively large amounts of methionine will growth of the organism in the absence of vitamin B₁₂. This interference can be readily detected in the pad-plate method by the faint diffuse growth zones exhibited by methionine. The pad-plate has also been used fully for the assay of proteogen with *Streptococcus* by reversal of propionate inhibition, for the assay of with *Lactobacillus bulgaricus*, and for the assay of *vitro* factor with *Leuconostoc citrovorum*. Preliminary experiments with the yeast, *Saccharomyces cerevisiae*, indicates that vitamin B₁₂, pantothenic acid, inositol biotin can be assayed with this organism using the plate procedure."

Laboratory of Microbiology,
Purdue University,
West Lafayette, Ind.

- 1014 FORD J. E.: *The microbiological assay of vitamin B₁₂*, Brit. J. Nutrition 6: 324-330, (No. 1) 1952.

BACTERIAL GROWTH, NUTRITION METABOLISM

1015. SHIVE, W., RAVEL, J. M., and EAKIN R. E.: *An interrelationship of thymidine and vitamin B₁₂*, *J. Am. Chem. Soc.* 70 2614-2615, July 1948 (Letter to Editor)

A medium containing enzymatic digest of casein is described which supports good growth of *Lactobacillus lactis* Dornier in the presence of liver extracts containing anti-pernicious anemia principles and can be used as an assay medium. With this medium, or with one containing clarified tomato juice in place of the enzymatic digest of casein, thymidine adequately replaced the liver extracts containing anti-pernicious anemia principles. Half-maximum stimulation of growth was obtained at a concentration of 1 to 3 mcg. thymidine per 10 cc. It therefore appears probable that vitamin B₁₂ functions in the biosynthesis of thymidine. When the medium containing tomato juice was utilized, the liver extract was replaced by as little as 1 cc. of sterile, aerated distilled water added aseptically to 10 cc. of medium, and this effect was enhanced by aseptic addition of ascorbic acid. When enzy-

matic digest of casein replaced the tomato juice, the aerated water was inactive, but 1 mg. of ascorbic in 1 cc. of sterile, aerated water per 10 cc. of medium adequately replaced the liver extracts.

1016. WRIGHT L. D., SKEGGS H. R., and HILL J. W.: *The ability of thymidine to replace vitamin B₁₂ as a growth factor for certain*, *J. Biol. Chem.* 175 475-476, Aug. 1948 (Letter to Editor)

Chemical investigation (*J. Am. Chem. Soc.* 70 1948) indicates that the crystalline compound functionally related to folic acid and isolated from liver is thymine deoxyriboside. For certain lactic acid bacteria, thymidine is able to replace the requirement for vitamin B₁₂. Studies on *Lactobacillus lactis* are which show that growth comparable to that obtained with liver is possible in the presence of 0.4 to 2.0 mcg. thymine per tube. Thymine is not active under conditions. The large amounts of thymidine

for optimal growth indicate that thymidine is not vitamin B₁₂. These results are interpreted as indicating that vitamin B₁₂ functions as a coenzyme in carrying out reactions concerned with conversion of thymine to thymidine, since in the presence of thymidine the vitamin is no longer required by the organism. This microbiological evidence also indicates that the biochemical defect in pernicious anemia may be the inability to synthesize certain nucleosides, particularly thymidine, from parent purines or pyrimidines, and that the curative effect of folic acid on the disease may be due to increased thymine synthesis, which by mass action effects yields more thymidine. This is further borne out by the effectiveness of large amounts of thymine in pernicious anemia which has been reported by other workers (Spies *et al.*, *South. M. J.* 89: 269, 1946).

- 1017 SHIVE, W., RAVEL, J. M., and HARDING W. M. An interrelationship of purines and vitamin B₁₂. *J. Biol. Chem.* 176: 991-992, Nov. 1948 (Letter to Editor)

Purines, or derivatives thereof, and thymidine are essential for the growth of *Lactobacillus lactis* Dorner in the absence of vitamin B₁₂. The purine requirement is less specific than the requirement of thymidine, which cannot be replaced by thymine. Guanylic acid was the most effective of single purines or derivatives, and mixtures of adenine and guanine or of hypoxanthine and guanine also were active. Three different concentrates of vitamin B₁₂ prepared by different processes replaced both purines and thymidine in the nutrition of the organism. These results indicate that vitamin B₁₂ is involved in the biosynthesis of purines (or their derivatives) as well as thymidine. These substances may in turn be involved in the biosynthesis of vitamin B₁₂.

Experiments on *Lactobacillus leichmannii* have shown that thymidine can replace the animal protein factor in the nutrition of the organism. This organism also requires folic acid for growth in a medium containing purines. The requirement for folic acid is replaced by thymine after a lag phase. Thymidine in the presence of folic acid replaces the animal protein factor and also slowly replaces both folic acid and the animal protein factor after a lag phase. Thus, independent functions are indicated for folic acid and for the animal protein factor which presumably is identical functionally with vitamin B₁₂ in the biosynthesis of thymine and thymidine.

1018. KITAY E., McNUTT W. S., and SNELL, E. E. The non-specificity of thymidine as a growth factor for lactic acid bacteria. *J. Biol. Chem.* 177: 993-994, Feb. 1949 (Letter to Editor)

For 11 of 13 microorganisms thymidine, hypoxanthine desoxyriboside, adenine desoxyriboside, or cytosine desoxyriboside were found to be equally active in promoting growth. Refined liver extract and desoxyribonucleic acid (DNA) were also active for many organisms. Ascorbic acid was active only rarely and at much higher concentrations. *Lactobacillus delbrueckii* 730 is unique among the organisms tested because it has a specific requirement for thymidine which can not be adequately replaced by the other desoxyribonucleosides or by low levels of liver extract. If the desoxyribonucleosides func-

tion by permitting synthesis of DNA, then in most of these organisms this is permitted by either the active factor of liver extract (presumably vitamin B₁₂) or by any one of four desoxyribonucleosides. It is possible that the desoxyribonucleosides function interchangeably by supplying their common portion, either as desoxyribose or as desoxyribose phosphate, for synthesis of DNA, and that this common portion may be formed by independent processes involving vitamin B₁₂.

University of Wisconsin
Madison, Wis.

- 1019 HOFF JØRGENSEN E.: Difference in growth promoting effect of desoxyribosides and vitamin B₁₂ on three strains of lactic acid bacteria. *J. Biol. Chem.* 176: 525-526, March 1949 (Letter to Editor)

In the course of a survey of the nutritional requirements of lactic acid bacteria of the genus *Thermobacterium*, a strain, *Thm. acidophilus* R26, was found which required thymidine. Neither a B₁₂ concentrate nor a commercial liver preparation could replace thymidine for this strain, but for *Thm. lactis* L, charcoal-treated tryptic casein digest was capable of replacing either thymidine or vitamin B₁₂. A third strain, *Thm. Juhart* L3, grew on a medium containing either thymidine or vitamin B₁₂, but tryptic casein digest was ineffective. Guanine desoxyriboside had the same effect as thymidine on all three strains.

1020. TOMARELLI, R. M., NORRIS R. F., and GYÖR GY P. Inability of vitamin B₁₂ to replace the desoxyriboside requirements of a *Lactobacillus bifidus*. *J. Biol. Chem.* 179: 485-486, May 1949 (Letter to Editor)

Contrary to the findings with other strains of *Lactobacillus*, vitamin B₁₂ even at high levels could not replace the desoxyribosides for the growth of a strain of *L. bifidus* isolated from the stool of a breast fed infant. Ribonucleic acid, thymine, and ascorbic acid, either alone or in combination with vitamin B₁₂, also were inactive. Growth was obtained on a chemically defined medium supplemented with an enzymatic digest of casein. It is suggested that the crude enzyme preparation contains a factor or factors much more active than the nucleosides. This is not crystalline vitamin B₁₂ but may be one of the other six entities demonstrated by Winsten and Eigen (Abstr. 1061) in samples possessing vitamin B₁₂ or animal protein factor activity.

- 1021 WELCH, A. D., and WILSON M. F. Mechanism of the growth-promoting effect of ascorbic acid on *Lactobacillus leichmannii* and the reduction of oxidation products of vitamin B₁₂. *Arch. Biochem.* 22: 486-489, July 1949

A digest of vitamin-free casein made with trypsin promotes vigorous growth of *Lactobacillus leichmannii* in the absence of added vitamin B₁₂ and the activity is increased by ascorbic acid treatment. The activity is only slightly altered by heating and is reduced one-half by autoclaving at pH 10. The remaining activity is increased by ascorbic acid treatment. Vitamin B₁₂ added prior to autoclaving is not completely inactivated and the residual activity is increased by ascorbic acid. Autoclaving vita-

min B_{12} in water at pH 10 reduces its microbiological activity by over 99 per cent. The apparent microbiological activity of ascorbic acid may be attributed to the occurrence of oxidation products of vitamin B_{12} in impure tryptin and in typically digested vitamin-free casein.

*Western Reserve University School of Medicine
Cleveland, Ohio*

1022. WRIGHT M. H. *Thymidine and vitamin B₁₂*. Science 110: 257-258, Sept. 9 1949

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*Laboratory of Medicine
Pratt-Lewis B. T.*

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University of Minnesota
Minneapolis, Minn.

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Yale University School of Medicine
New Haven, Conn.

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Leidos Laboratories
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Cornell University of Agriculture
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Laboratory
Food Research

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Corcoran Laboratory of Food Research
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1030. HENDLIN D., and RUGER, M. L. *The effect of cobalt on the microbial synthesis of LLD-active substances*, Science 111: 541-542, May 19 1950.

In an attempt to increase the microbial synthesis of vitamin B₁₂, cobalt—in the form of CO(NO₂)₂·6H₂O—was used to supplement the culture medium used. In experiments with *Streptomyces griseus*, it was found that the addition of cobalt ion (Co⁺⁺) to the basal medium increased the LLD titer about three-fold. An increase in vitamin B₁₂ production was also observed which was approximately parallel to the increase in LLD activity. As little as 1 to 2 ppm. of Co⁺⁺ produced maximal LLD activities. At levels of 20 to 50 ppm. of Co⁺⁺, toxic manifestations were noted, with a concomitant decrease in growth of the microorganisms and in LLD activity.

An extensive screening program has shown that large numbers of microorganisms synthesize LLD-active substances. In a study of some of these (including some isolated from cow rumen contents and cow manure) it was found that the addition of Co⁺⁺ (2 ppm.) resulted in a significant increase in the LLD titer of the broth.

Ward & Co., Inc.
Rochester, N. Y.

1031. EMERY W. B., LEES, K. A., and WALKER, A. D. *Observations on a growth factor for Leuconostoc citrovorum*, Biochem. J. 46: 572-574, May 1950.

Concentrates of a growth factor for *Leuconostoc citrovorum* contained in liver extracts and fermentation liquors for *S. griseus* have been prepared and purified. Vitamin B₁₂ was removed by an ion-exchange process. The factor is not identical with vitamin B₁₂ or B₁₂; it has no activity against pernicious anemia, but possesses leucocyte-stimulating activity.

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Eighteen strains of lactic acid bacteria, representative of six species, were tested for certain additional nutritional requirements. None of them grew in a medium complete with respect to known amino acids and synthetic vitamins and supplemented with tomato juice and an enzymatic digest of casein. All grew when thymidine was added to this medium. In most cases, thymidine could be replaced by certain other desoxyribosides. Several strains grew better with thymidine than with other desoxyribosides, and one (*Leuconostoc citrovorum* 8081) grew only with thymidine. Most organisms showed delayed growth with desoxyribonucleic acid.

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to previously autoclaved media, but only one-seventh as effective when it was added to the medium before sterilization. All organisms required similar amounts of the desoxyribosides for growth, but their requirements for vitamin B₁₂ varied.

University of Wisconsin
Madison, Wisc.

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In experiments on the nutritive requirements of lactic acid bacteria previously reported not to grow in media of known composition, 18 of the 28 cultures required thymidine, other desoxyribosides, or vitamin B₁₂ for growth.

University of Wisconsin
Madison, Wisc.

1034. DAVIS, B. D., and MINGIOLI, E. S.: *Mutants of Escherichia coli requiring methionine or vitamin B₁₂*, J. Bact. 60: 17-28, July 1950.

A number of mutants of *Escherichia coli* have been isolated that require vitamin B₁₂ or methionine. They do not respond to homocysteine, the immediate precursor of methionine, and mutants which do respond to homocysteine do not respond to B₁₂. Most of the mutants grow equally rapidly on B₁₂ or methionine, but two grow rapidly on methionine and slowly on B₁₂. Vitamin B₁₂ appears to be concerned with the methylation of homocysteine. The mutants are stable enough to be used for vitamin B₁₂ assay. The quantitative response to B₁₂ is greater and more prolonged in shaken than in unshaken tubes. With such assays it could be shown that suspensions of wild type *E. coli* rapidly absorb large amounts of vitamin B₁₂ from the medium.

Cornell University Medical College
New York, N. Y.

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When a solution of crystalline vitamin B₁₂ was subjected to a series of reducing agents (cysteine, ascorbic acid, thiocyanic acid, hydroquinone) between pH 4 and 7 at which range this vitamin is stable, a marked loss of the microbiological activity occurred. This loss can be attributed to the reducing power of the agents, but is not necessarily related to the disappearance of the intensity of the red color. The ATP activity of the solution was not reduced to the same extent as the microbiological activity.

Johns Hopkins University
Baltimore, Md.

1036. ROBBINS, W. J., HERVEY A., and STEBBINS, M. E.: *Studies on Euglena and vitamin B₁₂*, Science 112: 455, Oct. 20, 1950.

The use of *Euglena gracilis* var. *banillaris* in the bioassay of vitamin B₁₂ is reported. It was found that many bacteria and actinomycetes in the soil can synthesize vitamin B₁₂. Yeasts and filamentous fungi apparently are less commonly able to do so. When one part

of fresh soil was shaken with two parts of water the extracts contained amounts of vitamin B₁₂ similar to those in cow's milk. The roots of many higher plants contain vitamin B₁₂ in amounts ranging from 0.0002 to 0.01 mcg./Gm. of fresh material. It is believed that the vitamin B₁₂ is absorbed from soil solutions. A few stems have been shown to contain vitamin B₁₂ leaves and fruits did not. It was found that pond water on which *Euglena* develops regularly contained vitamin B₁₂ throughout the year in concentrations of 0.0001 to 0.001 mcg./cc. The pond mud was shown to contain bacteria and actinomyces capable of vitamin B₁₂ synthesis. The authors believe that the original source of vitamin B₁₂ in nature is the synthesizing activity of bacteria and actinomycetes, and not that of higher plants.

New York Botanical Garden
New York, N. Y.

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Attempts were made to induce dwarf strains of bacteria to form large colonies. For this purpose certain additions to plain nutrient agar (on which only tiny colonies were produced) were made. *Corynebacterium* produced large colonies when thiamine was added, *Alcaligenes faecalis* when thiamine plus vitamin B₁₂ was added, *Alcaligenes faecalis* when riboflavin was added, and *Shigella sonnei* when yeast extract was added. When again subcultured on plain nutrient agar the cells of large colonies of all the dwarf strains formed only tiny colonies.

University of Chicago
Chicago, Ill.

1038. REGE, D. V., and SREENIVASAN A. *Folic acid vitamin B₁₂ and nucleic acid synthesis in Lactobacillus casei*, Nature 166 1117 Dec. 30, 1950.

The effects of vitamin B₁₂ and folic acid on nucleic acid metabolism were investigated by growing *Lactobacillus casei* in media containing varying concentrations of these vitamins, singly or together. Folic acid was found to enhance the synthesis of deoxyribonucleic acid. This was also true of vitamin B₁₂, although the latter could not completely replace folic acid as a growth factor of *L. casei*. The most interesting observation, however was that there is a pronounced and more or less all-around additive effect of folic acid and vitamin B₁₂.

University of Bombay
Bombay, India

1039. WOOLLEY D. W. *Inhibition of synthesis of vitamin B₁₂ and of riboflavin by 1,2-dichloro-4,5-diaminobenzene in bacterial cultures*, Proc. Soc. Exper. Biol. & Med. 75: 745-746, Dec. 1950.

The amounts of vitamin B₁₂ and of riboflavin formed in cultures of *Bacillus megaterium* were decreased by 1,2-dichloro-4,5-diaminobenzene in quantities insufficient to inhibit growth. The production of vitamin B₁₂ was stimulated by the dimethyl analog of diaminobenzene. These facts are regarded as compatible with the previously expressed postulates that the dimethyldiamine is a precursor of riboflavin and of vitamin B₁₂, and that the

dichloro analog exerts its selective toxicity at least in part, by inhibition of biosynthetic processes in the formation of these vitamins.

Rockefeller Institute for Medical Research
New York, N. Y.

- 1040 WOOLLEY D. W. *Selective toxicity of 1,2-dichloro-4,5-diaminobenzene its relation to requirements for riboflavin and vitamin B₁₂*, J. Exper. Med. 93: 13-24 Jan. 1951

Authors' summary: "In a series of 26 species [of organisms] selected from widely differing classes, 1,2-dichloro-4,5-diaminobenzene was toxic to those which did not exhibit a nutritional need for riboflavin plus vitamin B₁₂. It failed to retard the growth of those which needed both of these vitamins. The compound was conceived as an antimetabolite of 1,2-dimethyl-4,5-diaminobenzene. This latter which is contained within the structures of the two vitamins, was pictured as a metabolic precursor of them. It was found to have very slight activity as either riboflavin or as vitamin B₁₂ for lactic acid bacteria and algae. The growth inhibiting action of the dichloro-diaminobenzene was overcome competitively by the dimethyldiaminobenzene, and also, to a lesser extent, by o-phenylenediamine. The toxicity was not influenced by additions of riboflavin plus vitamin B₁₂ except in the cases of two species, where the influence was slight. These facts were considered to support the idea that properly constructed analogs of a precursor of two or more essential participants in cell division may be able to circumvent the counteraction which the vitamin has been found to exert on an antimetabolite of its precursor. Alternate explanations of the observed data were likewise considered."

Rockefeller Institute for Medical Research
New York, N. Y.

1041. WEYGAND F., WACKER, A., and WIRTH, F. *Relations between vitamin B₁₂, purines and pyrimidines in connection with Lactobacillus leichmannii* 313 Z. Naturforschung 5b: 25-34 Jan. Feb. 1951.

The requirement of *L. leichmannii* for purines and pyrimidines has been determined. For optimal growth only guanine (I) and uracil are needed. In the absence of I or xanthine, adenine (II) inhibits the growth, stimulated by B₁₂ or deoxyribosides. It is concluded that the organism can not transform II into I. 4-Amino- and 5-methyl-4-amino-2-thiouracil, benzimidazole and its dimethyl, dibromo and dichloro derivatives also inhibit growth in the absence of II. This growth inhibition is competitively antagonized by II and its derivatives and noncompetitively by B₁₂.

1042. SHIVE, W., SIBLEY M. E., and ROGERS L. L. *Replacement of vitamin B₁₂ by deoxynucleotides in promoting growth of certain lactobacilli* J. Am. Chem. Soc. 73: 867-868, Feb. 1951 (in Notes)

The authors state, in part "Although vitamin B₁₂ is approximately 10,000 to 80,000 times as effective as the deoxynucleotides in stimulating growth of the lactobacilli in test tubes, the effect of the deoxynucleotides cannot be accounted for on the basis of contamination

1030. HENDLIN D., and RUGER, M. L.: *The effect of cobalt on the microbial synthesis of LLD-oxotic substances* Science 111 541-542, May 19 1950

In an attempt to increase the microbial synthesis of vitamin B₁₂, cobalt—in the form of CO(NO₃)₂·6H₂O—was used to supplement the culture medium used. In experiments with *Streptomyces griseus*, it was found that the addition of cobalt ion (Co⁺⁺) to the basal medium increased the LLD titer about three-fold. An increase in vitamin B₁₂ production was also observed which was approximately parallel to the increase in LLD activity. As little as 1 to 2 ppm. of Co⁺⁺ produced maximal LLD activities. At levels of 20 to 50 ppm. of Co⁺⁺ toxic manifestations were noted, with a concomitant decrease in growth of the microorganisms and in LLD activity.

An extensive screening program has shown that large numbers of microorganisms synthesize LLD-active substances. In a study of some of these (including some isolated from cow rumen contents and cow manure) it was found that the addition of Co⁺⁺ (2 ppm.) resulted in a significant increase in the LLD titer of the broth.

Novak & Co., Inc.
Rahway, N. J.

1031. EMERY W. B., LEES K. A., and WALKER, A. B.: *Observations on a growth factor for Leuconostoc citrovorum*, Biochem. J. 46 572-574 May 1950.

Concentrates of a growth factor for *Leuconostoc citrovorum* contained in liver extracts and fermentation liquors for *S. griseus* have been prepared and purified. Vitamin B₁₂ was removed by an ion-exchange process. The factor is not identical with vitamin B₁₂ or B₁₂. It has no activity against pernicious anemia, but possesses leukocyte-stimulating activity.

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Vitamin B₁₂ replaced thymidine (or other deoxyribosides) for many but not all of these organisms. *L. delbrueckii* 730 and *Lactobacillus acidophilus* 204 are examples of organisms that respond to thymidine but not to vitamin B₁₂.

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University of Wisconsin
Madison, Wis.

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University of Wisconsin
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Yale University
New Haven, Conn.

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University of Chicago
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Bombay, India

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Rochester Institute for Medical Research
New York, N. Y.

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with vitamin B₁₂, because the desoxynucleotides are inactive in the *Escherichia coli* assay are, relative to vitamin B₁₂, more effective in plate assays than in tube assays with the lactobacilli, and migrate differently on paper chromatograms."

University of Texas, and
Cotton Foundation for Research
Austin, Texas

1043. WOOLLEY D. W., and PRINGLE, A. *Structure-activity relationships among analogs of dimethyl-diaminobenzene as growth-inhibitors*, Federation Proc. 10: 272, March 1951.

"Because 1,2-dichloro-4,5-diaminobenzene, an analog of 1,2-dimethyl-4,5-diaminobenzene, has been shown to function as an antimetabolite of this precursor of riboflavin and of vitamin B₁₂, a further study of relatives of this metabolite has been undertaken. The dichloro-compound was a selectively toxic agent which affected only species requiring riboflavin and B₁₂ nutritionally. Furthermore, these 2 vitamins were unable to counteract the analog. Many structural relatives of the dimethyl-diaminobenzene were synthesized, several of them for the first time, and tested for potency against *Staphylococcus aureus* and mice. Alkylation of one amino-group, or replacement by nitro, abolished activity even when one or both of the methyl-groups was simultaneously exchanged for chlorine atoms. When one hydroxyl replaced one amino, active agents were formed, either when the methyls were untouched, or when they were exchanged for chlorines. Alkylation of the hydroxyl abolished activity. The nitrophenols formed by replacing the amino groups with hydroxyl and nitro were very potent, provided that at least one of the methyls was also exchanged for halogen. When the competitive antagonism with the dimethyl-diaminobenzene was tested, a similar relationship to that already established with analogs of phenylalanine (chloro-tyrosine, etc.) was found. The activity of the aminophenols was only partially reversed by the metabolite, and that of the nitrophenols not at all. Halogenation increased the tendency to non-competitive behaviour with the metabolite. Nevertheless, the amino- and nitro-phenols still actively inhibited the biosynthesis of B₁₂, just as the dichlorodiamine has been shown to do in growing bacterial cultures."

Escherichia Institute for Medical Research
New York, N. Y.

1044. HENDLIN D., and SOARS, M. H.: *Effect of 5,6-dimethylbenzimidazole and related compounds on the growth of L. lactis Dornier* Federation Proc. 10: 384, March 1951.

"5,6-Dimethylbenzimidazole has been reported as a degradation product obtained from vitamin B₁₂ on acid hydrolysis. The ability of 5,6-dimethylbenzimidazole and some related compounds to replace vitamin B₁₂ in the nutrition of the rat has been described. None of these compounds possessed vitamin B₁₂ activity for *Lactobacillus lactis* Dornier when tested at levels as high as 1 mg./ml. More than that, the benzimidazole compounds were found to inhibit markedly the growth of *L. lactis* in the presence of as much as 1 µg./ml. of vitamin B₁₂. The following compounds possessed inhibitory properties: 1,2-diamino-4,5-dimethylbenzene, 5-methyl-4,5-dimethyl and 5,6-dimethylbenzimidazole. No inhibition of growth was obtained with benzimidazole, 1-methyl-

2-methyl, 4-methyl, and 2,5-dimethylbenzimidazole at levels as high as 1 mg./ml. The inhibition obtained with 1,2-diamino-4,5-dimethylbenzene was competitively antagonized by vitamin B₁₂ over a narrow range of inhibitor. This was not the case with 5,6-dimethylbenzimidazole. Higher levels of the substituted diaminobenzene produced inhibition which could not be reversed by the known B vitamins, purines, pyrimidines, nucleosides and nucleotides, singly or in combination."

Merck & Co., Inc.
Rahway, N. J.

1045. DAVIS R. L., LAYTON, L. L., and CHOW B. F.: *Uptake of radioactive vitamin B₁₂ by bacteria in single and mixed cultures*, Federation Proc. 10: 380, March 1951.

"In view of the lack of agreement as to the role of the intestinal flora with respect to various nutrients in the gut, an *in vitro* study of vitamin B₁₂ metabolism of certain bacteria is being made. Culture media containing vitamin B₁₂ labeled with Co⁶⁰ were inoculated with various types of bacteria. After incubation, the resultant bacterial cultures were centrifuged, the washed cells and media were assayed for radioactive Co⁶⁰ as CoSO₄ and CoS respectively using a Geiger-Müller counter. Aliquot samples of media (80 ml.) containing 4 µg. vitamin B₁₂ with total activity of 60 cpm were inoculated with *L. leichmannii*, *E. coli*, *S. lactis* R and *L. arabinosus*. After 45 hours incubation, the cells were separated, washed and assayed for radioactivity. It was found that about one-half of the added activity was present in the first 3 organisms mentioned above and a negligible amount in the last one. Competitive systems of bacteria were also studied: the types of organisms cultured were separated by cellophane membranes through which vitamin B₁₂ was dializable. In one such system in which *L. leichmannii* and *L. arabinosus* were separated by cellophane and 6 µg. labeled vitamin B₁₂ included in the media, subsequent assay for radioactivity of the grown cells and media demonstrated that *L. leichmannii* absorbed the same proportion of this activity as when cultured alone. Data on the behavior of other competitive bacterial systems for radioactive vitamin B₁₂ are also presented as well as methods for the isolation of Co⁶⁰."

Johns Hopkins University
Baltimore, Md.

1046. OGINSKY E. L., SMITH, P. H., TONHAZY N. E., UMBREIT W. W., LICHTSTEIN R. C., and CARSON S. F. *The influence of vitamin B₁₂ on oxidation by a mutant strain of Escherichia coli*, J. Bact. 61: 581-590, May 1951.

The influence of vitamin B₁₂ on the rate of oxidation of a number of substrates by resting cell suspensions was studied with a mutant strain of *Escherichia coli* that required methionine and the vitamin for growth. The vitamin markedly increased the oxidation rate. In the course of the experiments it was found that 36 mcg. of streptomycin per cc. inhibited the oxidation of acetate in the presence or absence of B₁₂ when added with the acetate. However streptomycin had no effect on the oxidation if added an hour after the oxidation had begun.

North Institute for Therapeutic Research
Rahway, N. J.
Oak Ridge National Laboratories
Oak Ridge, Tenn.

1047. LOCHHEAD A. G., and THIXTON R. H.: *Vitamin B₁₂ as a growth factor for soil bacteria*, Nature 167 1034 June 23, 1951.

In the course of taxonomic and physiological studies of bacteria incapable of growth in the yeast extract medium, but showing abundant growth upon the addition of soil extract, the ability of vitamin B₁₂ to replace soil extract as growth-promoting factor was determined. Of 534 bacterial strains, isolated on a non-selective basis from field soil, 75 strains, or 14.0% fell into this special group. Replacement of soil extract by vitamin B₁₂ added to give a concentration of 2 mgm. per ml., permitted optimal growth of 26.7% and sub-maximal growth of 28.0% of this group. In the case of the remainder vitamin B₁₂ gave no growth response.

Department of Agriculture
Ottawa, Canada

1048. McRORIE, R. A., and WILLIAMS W. L. *Studies on the relationship between the Lactobacillus bulgaricus factor and pantothenic acid*, J. Bact. 61 737-745, June 1951

"All 27 strains of the lactic acid bacteria studied utilize LAF [Lactobacillus bulgaricus factor] under the conditions tested. Twenty five strains appear to require LAF for growth, i.e., LAF is more active for these organisms than an equivalent amount of pantothenate. These include all 12 strains of Lactobacillus bulgaricus tested, 11 out of 11 strains of Lactobacillus acidophilus that grow on the LAF basal medium supplemented with vitamin B₁₂, and the single strain of Lactobacillus delbrueckii tested. For Lactobacillus arabinosus LAF is only one-twelfth as active as would be expected from its pantothenate content, and for Lactobacillus casei the response is approximately equal to the pantothenate content of LAF. The yeast, Saccharomyces carlsbergensis, failed to respond to LAF.

"The pantothenate fragments, pantoyl lactone and pantolic acid, had no effect on the lactic acid bacteria tested, either alone or in combination with pantothenate. β-Alanine appears to enhance the utilization of pantothenate for these organisms.

"Orotic acid was found to be only stimulatory for all strains of L. bulgaricus except for L. bulgaricus 09 previously reported to require this substance (Wright, 1950b).

"It is suggested that the various forms of LAF are normal intermediates in the biosynthesis of coenzyme A from pantothenic acid."

North Carolina Agricultural Experiment Station
Raleigh, N. C.
Ludvik Lohmeyer
Paul Riser, Jr.

1049. LITTLE, P. A., OLESON J. J., and WILLIAMS, J. H. *Growth studies on Polytomella agilis* Proc. Soc. Exper. Biol. & Med. 78 510-513, Nov. 1951.

Authors' summary: "The nutritional requirements of a strain of Polytomella agilis were investigated. Ethanol, butanol, acetate, propionate or butyrate were utilized as carbon sources. Glutamine was adequate as a sole source of nitrogen. Thiamine and vit. B₁₂ were stimulatory

A simplified medium, adequate for continuous subculture of this organism, is presented."

Ludvik Lohmeyer
Paul Riser, Jr.

1050. LARDY H. A.: *Metabolic functions of the micro-catalytic B vitamins* Rec. Chem. Progr. 12 9-18 Winter 1951

Biochemists have developed the theory that the water-soluble B vitamins exert their catalytic influence in living cells by being converted to compounds which form the prosthetic or functional group on specific enzymes. Thus, the functional forms for these vitamins are for thiamine, thiamine pyrophosphate (cocarboxylase) for riboflavin, flavin monophosphate and flavin-adenine dinucleotide for niacin, diphosphopyridine nucleotide (coenzyme) and triphosphopyridine nucleotide for pyridoxal, pyridoxal phosphate (codecarboxylase) for pantothenic acid, coenzyme A (a pantothenate-containing dinucleotide) for folic acid, citrovorum factor or derivatives, for vitamin B₁₂, and for biotin, the functional forms have not been fully elucidated.

The first five of these vitamins, which function in the metabolic utilization of the major classes of foodstuffs and in the decarboxylation, transamination and decarboxylation of the amino acids, are classified as macro catalysts, and the last three, which function in the synthesis of comparatively small quantities of cellular building blocks, are classified as micro catalysts. Much less is known concerning the structure of the coenzyme form of these micro catalysts, but vitamin B₁₂ may already be in the coenzyme form since phosphate is a part of its molecule. All these micro vitamins seem to be involved in the metabolic transformation of compounds containing a single carbon atom. The metabolic functions of biotin and of the folic acid group of vitamins and vitamin B₁₂ are discussed.

University of Wisconsin
Madison, Wis.

1051. VILLELA, G. G., and ABREU L. A. *Lack of inhibition of growth of Euglena gracilis by vitamin B₁₂ oxidation product*, Science 115 205, Feb. 22, 1952.

This experiment shows that the oxidation product resulting when vitamin B₁₂ is treated with strong acid and hydrogen peroxide is not a competitive antagonist to vitamin B₁₂ when assayed with Euglena gracilis. The antagonism has been shown to exist when Lactobacillus leichmannii 4797 is the test organism.

Instituto Osvaldo Cruz
Rio de Janeiro, Brazil

1052. DAVIS, R. L., LAYTON L. L., and CHOW B. F. *Uptake of radioactive vitamin B₁₂ by various microorganisms*, Proc. Soc. Exper. Biol. & Med. 79 273-276, Feb. 1952.

The incorporation of vitamin B₁₂ into bacterial cells of a species requiring this vitamin for growth (Lactobacillus leichmannii) a species which can grow without an exogenous source of this vitamin (L. arabinosus) and one which requires it only during the initial phase of growth (L. lactis Dornier) was studied. Seven other species (E. coli, S. lactis, B. subtilis, P. mirabilis,

mycoides, *Esch. freundii*, *S. dohsonii*) were included in the study. The microorganisms were grown in media containing radioactive vitamin B₁₂ tagged with Co⁶⁰. Radioactivity was found in the cells of all the species studied except *L. strubis*. It was estimated that approximately 8 molecules of the tagged vitamin B₁₂ united with each *L. leichmannii* cell, the number uniting with the cells of the other organisms varied from 12 to 60 and was not related to their growth requirement of vitamin B₁₂; organisms which required no vitamin B₁₂ were capable of incorporating it. No radioactivity appeared in the cells when radioactive cobaltous chloride was used instead of radioactive vitamin B₁₂. To investigate the fate of the tagged vitamin in a system of competitive organisms such as is found in the intestinal tract, two microorganisms were separated by a cellophane membrane which was readily permeable to the vitamin. Radioactivity entered the cells of both organisms in essentially the same amounts as when each species was grown alone.

Johns Hopkins University
Baltimore, Md.

- 1053 OGINSKY E. *Uptake of vitamin B₁₂ by Esch. coli*. Arch. Biochem. & Biophysics 36: 71-79 March 1952 (abstr. Blood 7: 857 Aug. 1952)

"Cells of a vitamin B₁₂ deficient mutant strain of *E. coli* are capable of absorbing radioactive vitamin B₁₂ labeled with Co⁶⁰ in excess of amounts required for growth or oxidation. This observation agrees with the finding of Klotzschek and Hemlin using *Lactobacillus lactis* that these bacteria take up considerably more vitamin than is required for growth.

"In the presence of glucose, the absorption rate of deficient cells was decreased. No exchange of intracellular B₁₂ in respiring cells could be demonstrated."

New York Institute for Therapeutic Research
Rensselaer, N. Y.

- 1054 DAVIS, R. L., and CHOW B. F.: *Determination of vitamin B₁₂ by means of its combination with L. leichmannii resting cells*, Federation Proc. 11: 466, March 1952.

"Both the growing and 'resting' cell of *L. leichmannii* (ATCC 4947) can incorporate vitamin B₁₂ from the environment; the latter in greater magnitude. These findings enabled us to develop some techniques useful in the rapid concentration and estimation of both radioactive and non-radioactive vitamin B₁₂ present in a variety of biological fluids. This was accomplished by the addition of a suspension of 'resting' cells of *L. leichmannii* to given volumes of the test fluid. After standing at room temperature for approximately 30 minutes the microorganisms, which by then would have taken up quantitatively the vitamin B₁₂, were centrifuged and washed with isotonic saline solution. With samples containing radioactive vitamin B₁₂, the washed organisms were transferred to a planchet and the radioactivity measured. To estimate the non-radioactive vitamin B₁₂, two quantitative procedures were developed. 1) The microbiological assay in which the washed cells were suspended in normal saline solution and heated at 60° C. for 1 hour and the resulting suspension was assayed microbiologically for vitamin B₁₂.

2) an isotope-dilution method, in which use was made of a standard curve relating the uptake of radioactivity and the total quantity of vitamin B₁₂ consisting of a constant amount of radioactive and increasing amounts of non-radioactive vitamin B₁₂. The uptake of vitamin B₁₂ by *L. leichmannii* was effective over a wide range of pH's but was diminished at salt concentration above isotonicity. The advantages of our procedure lie in its rapidity and comparative lack of inhibitors."

Johns Hopkins University
Baltimore, Md.

- 1055 DUBNOFF J. W. *Role of B₁₂ in methionine synthesis in E. coli mutants 115-3* Federation Proc. 11: 205, March 1952.

"Homocysteine, glutathione or cysteine partially replaces the B₁₂ requirement of the *E. coli* mutant 115-3. The growth promoting ability of these compounds is markedly enhanced by small amounts of B₁₂. This second requirement for B₁₂ is at least partially replaced by the specific methyl donor dimethyl-β-propiophthetin provided homocysteine or its thiolactone is used to replace the major B₁₂ requirement. Other methyl compounds such as choline, betaine, dimethylthetin, and dimethyl-γ-butyrothetin which are active in animals are completely inactive in this mutant."

California Institute of Technology
Pasadena, Calif.

- 1056 ROBBINS W. J., HERVEY A., and STEBBINS M. E. *Bacteria in air make vitamin B-12 from dust*, Science News Letter 62: 296, Nov. 8, 1952.

Bacteria in the air can synthesize vitamin B₁₂ from dust. This was shown when pure water was first exposed to air for two months and then seeded with *Engelmannia*. *Engelmannia* are one-celled green plants that grow only in the presence of vitamin B₁₂.

"As the *Engelmannia* thrived, [the investigators] deduced that B-12 was present. They were able to measure the vitamin content of the water by the amount of *Engelmannia* growth, and found two millionths of a millionth of a gram (a micro-micro-gram) of the vitamin in each milliliter of water.

"As the water was completely sterile and pure at the beginning of the experiment, the scientists were able to deduce that bacteria from the air which fell into the water had converted food material present as dust in the air into the vitamin."

New York Botanical Garden
New York, N. Y.

- 1057 DAVIS, R. L., and CHOW B. F.: *Compounds inhibiting uptake of vitamin B₁₂ by "resting" bacterial cells*, Federation Proc. 12: 440, March 1953.

"Compounds such as nucleic acids, mucopolysaccharides and others can reduce the uptake of environmental vitamin B₁₂ by the 'resting' cells of *Lactobacillus leichmannii* ATCC 4792(LL). The extent of this inhibition of uptake is dependent on the quantity of the test substance, the chemical structure, in some instances the polymerized state, and finally on the processing of the resting"

cells. The order of addition is likewise of importance; the most effective sequence being the test substance, resting cells, and vitamin B₁₂. The most inhibitory substances, heparin and other sulfonated compounds are capable of reducing by 50% the uptake of 20 mμ vitamin B₁₂ in 2-5 mcg. quantities, in a system containing approximately 2×10^8 resting cells. Two strains of *Escherichia coli*, namely 113-2 and Waksman, whose uptake for vitamin B₁₂ was demonstrated by B. D. Davis and Oginsky responded differently than L., qualitatively and quantitatively to these compounds. For example, the uptake of vitamin B₁₂ by both strains of *E. coli* was unchanged by heparin. Methylene blue, laurylthyleneoxide and triiodomethylbenzimidazoleacetate were inhibitory only to *E. coli*, whereas neomycin, polyglutamic acid (ALW 8000) and hyamine were inhibitory to all. Since consistent and reproducible responses to the inhibitors were noted, a satisfactory procedure was established to measure these compounds in unknown samples by comparing with a standard curve, the amount of reduction of a given dose of vitamin B₁₂. Other applications and implications on these observations will be reviewed."

Johns Hopkins University
Baltimore, Md.

1058. DUBNOFF J. W.: Influence of vitamin B₁₂ on enzyme activity in *E. coli* 113-3. Federation Proc. 12: 198, March 1953.

"The influence of B₁₂ on the oxidation of a wide variety of substrates first demonstrated by Oginsky et al. is independent of the cytochrome system since the oxidation measured by the reduction of methylene blue and tetrazolium is similarly affected. Certain preparations of the mutant which do not show the B₁₂ effect will respond to the vitamin if reduced glutathione is added. Cysteine will not replace glutathione. The aging process usually required to show a response to B₁₂ results in a decrease in enzymatic activity. Data will be presented suggesting that this decrease in activity is related to a decrease in

protein sulphydryl as measured by the ferricyanide method and that reduced glutathione, B₁₂ and certain substrates form a system that increases protein sulphydryl in this mutant."

California Institute of Technology
Pasadena, Calif.

1059. KALAN E., and CEITHAM, J. Synthesis of methionine in *E. coli*, Federation Proc. 12: 228 March 1953.

By the use of biochemical mutants, vitamin B₁₂ has been implicated in the biosynthesis of methionine in *E. coli* W ATCC 9637 (DAVIS AND MINICOLI, J. Bact. 60: 17 1950) [See Abstr 1034]. A series of methionine requiring mutants has been isolated in our laboratory. These were studied on agar plates which contained the minimal medium supplemented with methionine or various possible methionine precursors, individually, and in combination with vitamin B₁₂. It was found that these mutants could be classified into four main categories. The mutants in the first category grow only in the presence of methionine. Those in the second group require either methionine or vitamin B₁₂ for growth. The third category consists of those mutants which exhibit optimal growth in the presence of the following supplements: methionine, homocysteine, homocystine or cystathionine. Addition of B₁₂ effects no stimulation of growth. The mutants of the fourth group are similar in their requirements to those of the third group. Moreover some of these mutants can grow on alanine, homoserine, valine, isoleucine or alpha amino butyric acid. However optimal growth occurs only in the case of methionine. On the other effective supplements, growth is slight, but when B₁₂ is added, growth is stimulated. These results support the view that B₁₂ is involved in methionine synthesis. They also indicate possible amino acid precursors for the 4-carbon moiety of the methionine molecule."

University of Chicago
Chicago, Ill.

ASSAYS OF VITAMIN B₁₂ ACTIVITY AND CONTENT

CHROMATOGRAPHIC

1060. TISHKOFF G. H., ZAFFARONI, A., and TESLUK, H.: Purified liver extract chemical nature as determined by paper partition chromatography. J. Biol. Chem. 175: 857-862, Sept. 1948.

Studies of a liver extract containing a high concentration of the anti-pernicious anemia factor revealed the presence of one or more polypeptides of high molecular weight, some free amino acids and riboflavin. Folic acid and xanthopterin were not detected. The polypeptide which is presumably the active constituent, was separated from the free amino acids and the amino acids obtained by hydrolysis of the polypeptide were identified by paper chromatography.

1061. WINSTEN W. A., and EIGEN E.: Paper chromatography of vitamin B₁₂ and related bacterial growth factors, Federation Proc. 8: 265-266, March 1949; J. Biol. Chem. 177: 989-990, Feb. 1949.

The paper chromatographic procedure for separating the six alternate growth factors for *Lactobacillus leichmannii*, two of which are found in vitamin B₁₂ concentrates, is reported, as well as the method of recognizing the positions of the several growth factors on a chromatogram by the use of *L. leichmannii* as a microbiologic indicator. The slow movement of the two forms of vitamin B₁₂ on a paper chromatogram with n-butanol as the mobile phase permits the separation of these entities from the faster moving substitute growth factors. The isolated vitamin B₁₂ entities may then be assayed by the usual tube assay procedure.

1062. YACOWITZ, H., NORRIS L. C., and HEUSER, G. F.: An improved method for the microbiological assay of growth factors on paper chromatograms, Proc. Soc. Exper. Biol. & Med. 71: 872-874, July 1949.

A modification of the method of Winston and Eigen (Abstr 1061) for the microbiologic assay of nutritional growth factor is described. This modification permits

both qualitative and quantitative assay by means of paper chromatograms, and avoids the interference of thymidine in the assay of vitamin B₁₂. This method has been found to be sensitive to 0.1 millimicrogram of vitamin B₁₂ present in the developed chromatogram. *Lactobacillus leichmannii* is used as the assay organism.

- 1063 BORSOOK, H., DEASY, C. L., HAAGEN-SMIT, A. J., KEIGHLEY, G., and LOWY, P. H. A convenient quick method of obtaining vitamin B₁₂ concentrate, *Science* 110 528-529 Nov 18, 1949

The nonprotein filtrate of liver homogenate (proteins coagulated by boiling at pH 5) chromatographed on starch columns gave a reddish brown fraction in the first portions of the effluent. The method offers a way of obtaining B₁₂-active material from commercially available sources, which already contain the activity in a conveniently small volume.

California Institute of Technology
Pasadena, Calif.

- 1064 WINSTEN, W. A., and EIGEN, E. Paper chromatography of vitamin B₁₂ and related bacterial growth factors, *J. Biol. Chem.* 181 109-120, Nov 1949

A paper chromatographic procedure for separating vitamin B₁₂ and related growth factors is described. In addition to vitamin B₁₂, five other substitute growth factors of *L. leichmannii* 513 have been recognized. They are desoxyribosides.

- 1065 WIJNENGA, H. G., LENS, J., and MIDDELBECK, A. Some properties of vitamin B₁₂, *Chem. Weekblad.* 45: 342-343, 1949 (abstr. C. A. 43 9193/ Nov 23, 1949)

"Cryst. vitamin B₁₂ (I) (yield, 25 mg. per ton liver) had 3.8 ± 0.4% Co and absorption coeff. (log e % cm.) 280 mμ, 2.11 360 mμ, 2.26. Impure I contg. 0.45 ± 0.04% Co had absorption coeff. 290 mμ, 2.20 360 mμ, 2.40 550 mμ, 1.86 detd. with a Beckman spectrophotometer. The following values were obtained with a Kipp instrument using the same soln.: 80 mμ, 2.44 360 mμ, 2.50 510 mμ, 2.12. The observation that impure I contg. 0.45% Co has absorption max. at the same wave length as pure I, and that the impure prep. has a higher absorption coeff. than pure I indicates that the abs. coeff.-wave length curve cannot be used as an index of purity of I. In contrast with the findings of Ruckes, *et al.*, a 50% soln. of pure I in 0.1 N HCl was stable, as shown by no change in the absorption spectrum and no liberation of free Co, on 8 days standing. The unchanged absorption spectrum is in contrast with the report of E. Lester Smith

but this variance may be due to the still inexplicable result obtained in the following expts. A soln. of I was aerated for 1.5 hrs. At wave lengths below 300 mμ a total end absorption occurred and the absorption max. at 290 mμ had disappeared, without affecting the absorption at 360 mμ. Chromatographic absorption of the aerated I on neutral Al₂O₃ gave 2 fractions, each of which had absorption max. at 290 mμ. This was attributed to oxidation until the same result was obtained by passing CO₂ through the soln. Heating I in distd. H₂O (pH 5.3) results in formation of a yellow-brown color"

1066. SHAW, G. E. Paper chromatography of liver extracts, *Biochem. J.* 44 iv 1949

A paper chromatographic method for the assay of vitamin B₁₂ is described.

- 1067 CUTHBERTSON, W. F. J., and SMITH, E. L. Chromatography of the vitamin B₁₂ group of factors, *Biochem. J.* 44 v-vi, 1949

"The two red substances present in liver extract (one probably identical with vitamin B₁₂) thymidine and a fourth unidentified factor can be demonstrated by a combination of partition chromatography on paper with microbiological assay on a solid medium."

1068. SMITH, E. L., and CUTHBERTSON, W. F. J. Paper chromatography of the vitamin B₁₂ group of factors *Biochem. J.* 45 xli, 1949 (in Soc. Proc.)

All five naturally occurring purine and pyrimidine desoxyribosides show growth activity with *L. lactis* Dörner and their presence in extracts from liver and from *Streptomyces griseus* interferes with the plate assay process for vitamin B₁₂. A method of separating them by paper chromatography prior to B₁₂ assay is indicated.

- 1069 SMITH, E. L., CUTHBERTSON, W. F. J., WALKER, A., and LEES, K. A.: Partition chromatography of the vitamin B₁₂ group of factors, *Federation Proc.* 9 230, March 1950.

1070. WOODRUFF, H. B., and FOSTER, J. C.: Assays for vitamin B₁₂ and vitamin B₁₂ by paper strip chromatography *J. Biol. Chem.* 183 569-576, April 1950.

Authors summary: "A method has been presented for quantitative estimation of vitamins B₁₂ and B₁₂ combining microbiological assay and paper strip chromatography. A small corrective factor is required to compensate for conversion of vitamin B₁₂ to vitamin B₁₂ which may take place during the analysis. The method has been applied to microbiological fermentation broths and to injectable liver extracts (U.S.P.) for the analysis for vitamin B₁₂ type materials. No quantitative relationship was found between the amount of these vitamins contained in the samples and the *Lactobacillus lactis* titrimetric assay of the samples."

Woods H. C. Jr.
Baltimore, Md.

- 1071 FANTES, K. H., IRELAND, D. M., and GREEN, N.: A colorimetric assay method for vitamin B₁₂, *Biochem. J.* 46 xxxiv-xxxv May 1950 (in Soc. Proc.)

A colorimetric assay for vitamin B₁₂ is described which is capable of measuring about 40 mcg. in not more than 2 cc. Related cobalt-containing factors of the vitamin B₁₂ complex, such as vitamin B₁₂, behave like vitamin B₁₂ itself in this procedure.

Chas. Laboratories
Cambridge and Bedford, Cambs
England

1072. CHARGAFF E., LEVINE, C., GREEN C., and KREAM J. C.: Study of some constituents of vitamin B₁₂. *Experientia* 6: 229-231 June 1950

The occurrence of nucleic acids and the content of the amino compound in vitamin B₁₂ has been studied by paper chromatography. Neither purine nor pyrimidine or desoxyribose was found to be present. The amino derivative (calculated as 2-amino-1-propanol) was found to be present in a proportion of 2 mols per one mol of phosphorus.

Columbia University
New York, N. Y.

1073. PICKEN J. C., JR., and BAURIEDL, W. R.: Comparative bioautography of vitamin B₁₂ and related growth factors using *Escherichia gracilis* and *Lactobacillus leichmannii*. *Proc. Soc. Exper. Biol. Med.* 75: 511-515, Nov 1950.

A number of preparations to which *Escherichia gracilis* or *Lactobacillus leichmannii* has been reported to give a quantitative growth response (crystalline vitamin B₁₂, liver extract, corn steep liquor, desoxyribonucleic acid) were subjected, in duplicate, to paper chromatography in order to separate different factors. One of each pair of paper strips was then placed in contact for about five minutes with an agar plate seeded with *E. gracilis* and the other with a plate seeded with *L. leichmannii*. The plates were then incubated, and the growth response of the two organisms on different concentrations of the tested preparations was compared by means of photographs and graphs (bioautographs). The least amount of crystalline vitamin B₁₂ that would give definite visible growth when applied to the plate on a 1 cm² piece of filter paper was 0.01 mcg. for *E. gracilis* and approximately 0.5 mcg. for *L. leichmannii*. *E. gracilis* responded only in the area of the slower moving factors of the liver extract, where vitamin B₁₂ is expected to appear while *L. leichmannii* responded to three more rapidly moving factors in addition to the slow-moving factor. There were indications that the fastest moving factor was thymidine. *E. gracilis* did not respond to corn steep liquor while *L. leichmannii* responded to four moving factors. *E. gracilis* did not respond to any of the desoxyribonucleic acid preparations unless vitamin B₁₂ was added, in which case growth occurred in the area of the slow moving factors. *L. leichmannii* demonstrated the existence of a slow-moving factor or factors in the untreated desoxyribonucleic acid preparations. Since these factors promoted the growth of one organism but not of the other they cannot be considered identical with crystalline vitamin B₁₂. Thus the comparative bioautograph method may be useful in studying the slow-moving factors, as a qualitative method of indicating activities present in preparations which are to be assayed for vitamin B₁₂, and as a guide in separation and purification procedures.

Ann. Entom. College
Ann. Entom.

1074. KOCHER, V., KARRER, R., and MÖLLER, H. R.: Paper chromatography of vitamin B₁₂ and desoxyribonucleosides with phenol, n-butanol and collidine. *Z. Vitaminforsch.* 21: 403-409 1950.

St. Jakob Medical Laboratory
Basel

1075. VEER W. L. C., EDELHAUSEN J. H., WIJ MINGA H. G., and LENS, J.: Vitamin B₁₂. I The relation between vitamin B₁₂ and B_{12m}. *Biochim. Biophys. Acta* 6: 225-228, 1950

The spectrum of vitamin B₁₂ remains unchanged in mildly acidic solution if kept in the dark; on exposure to light it is transformed into B_{12m}. An irradiated solution of B_{12m} (pH=4) when kept in the dark, shows slow reversal to B₁₂. Addition of KCN to B_{12m} effects rapid conversion to B₁₂. B₁₂ is either a cyan complex or it has a spectrum which is identical with a B_{12m}-cyan complex.

1076. WIJ MINGA, H. G., VEER, W. L. C., and LENS, J.: Vitamin B₁₂. II The influence of HCN on some factors of the vitamin B₁₂ group. *Biochim. Biophys. Acta* 6: 229-236, 1950.

The absorption spectra (visible and ultraviolet) of vitamin B_{12m} and B₁₂ are identical and the two forms cannot be distinguished by chromatography (n-butanol solvent).

1077. HOLDSWORTH, E. S., and FORD J. E.: Differentiation of vitamin B₁₂ active compounds by ionophoresis and microbiological assay. *Nature* 171: 148-150, 1953 (abstr. *Blood* 4: 487 May 1953)

"There are several compounds related to vitamin B₁₂ but not in the cobalamin series. Using paper chromatography factor A, vitamin B_{12m}, and pseudo-vitamins B₁₂ and B_{12m} could not be separated with the solvents usually employed. The ionophoresis of these substances was tested. Results with veronal/acetate buffer were not helpful, but where 0.5 N acetic acid was used, with 0.01 per cent potassium cyanide, five substances could be distinguished. These, with their mobilities expressed as cm²V⁻¹ sec⁻¹ were factor A (vitamin B_{12m}) 3.9 × 10⁻⁴; factor B, 5.0 × 10⁻⁴; factor C, 1.4 × 10⁻⁴; pseudo-vitamin B₁₂, 1.5 × 10⁻⁴; vitamin B₁₂, zero mobility.

"The growth activities of these and other factors for micro-organisms were compared. B. coli (tube assay), B. coli (plate assay) and L. leichmannii being used. Factor A and vitamin B_{12m} have similar properties, as have pseudo-vitamins B₁₂ and B_{12m}. The two groups differ markedly in microbiologic activities and there are further differences with factors B, WR, and C. Factor WR is probably a mixture that includes inhibitors for the growth of B. coli."

University of Reading
Reading, England

VALENCE; MAGNETIC PROPERTIES

1078. LALAND P., and CLOSS K.: Formation of tri valent cobalt complexes in protein hydrolysates. *Nature* 163: 565, April 9 1949 (Letter to Editor)
1079. GRÜN F., and MENASSE, R.: Determination of the magnetic susceptibility of vitamin B₁₂. *Experientia* 6: 263-264, July 15, 1950.

Measurements of the magnetic susceptibility of vitamin B₁₂ have shown it to be diamagnetic.

Microbiologic measurement of the vitamin B₁₂ activity of seven samples of liver extracts for oral administration showed a wide variation in activity. The daily dose of the liver extracts is 45 cc., and represents a vitamin B₁₂ activity equivalent to 30 to 140 mcg. per day. This is considerably more than the 5 mcg. doses of crystalline vitamin B₁₂ which have been found to be effective when given orally together with gastric juice to pernicious anemia patients.

S. E. Munnick, Jr. Co.
Bristol, Tenn.

1098. TRENNER, N. R., BUHS, R. P., BACHER, F. A., and GAKENHEIMER, W. C.: *A note concerning the incompatibility of vitamin B₁₂ and ascorbic acid*, J. Am. Pharm. A. (Sc. Ed.) 39 361, June 1950.

The incompatibility of mixtures of vitamin B₁₂ and ascorbic acid has been reported. This incompatibility has been investigated further. The existence of B₁₂ analogs possibly has considerable significance in relation to the compatibility of certain vitamin B₁₂ concentrates and ascorbic acid. Highly purified crystalline vitamin B₁₂ preparations show a much higher degree of stability in the presence of pure ascorbic acid than previously reported.

Examination of a number of commercial vitamin B₁₂ preparations for ascorbic acid compatibility revealed that they vary greatly in this respect. These results are interpreted as evidence of the presence, in varying amount, of ascorbic acid-incompatible analogs of vitamin B₁₂ in some commercial preparations.

March & Co., Inc.
Rochester, N. Y.

1099. HARTLEY F., STROSS, P., and STUCKEY R. E.: *Some pharmaceutical aspects of vitamin B₁₂*, J. Pharm. and Pharmacol. 2 648-659 Oct. 1950.
1100. HERRNUNG G.: *Quantitative method for determination of vitamin B₁₂ content of commercial liver preparations*, Folia haematol. 70 110-1950.
1101. BOXER, G. E., and RICKARDS, J. C.: *Chemical determination of vitamin B₁₂. IV Assay of vitamin B₁₂ in multivitamin preparations and biological materials*, Arch. Biochem. 30 392-401, Feb. 1951.

The colorimetric method for determining vitamin B₁₂ (cyanocobalamin) has been applied to certain multivitamin preparations, liver and microbial concentrates, urine, gastric juice, and aqueous liver extracts. The determination of cyanide released after illumination with visible light proved to be specific for vitamin B₁₂ in the substrates investigated. The daily excretion of cyanide and vitamin B₁₂ in the urine was determined in 2 subjects. Cyanide excretion was rather constant and did not increase when a large dose of vitamin B₁₂ was injected. Vitamin B₁₂ excretion was irregular but some of the vitamin was found at all times in the urine. There was prompt excretion of a large injected dose of vitamin B₁₂, indicating that there was no need of, or facilities for the storage of the vitamin.

March & Co., Inc.
Rochester, N. Y.

1102. BOXER, G. E.: *Chemical assay method for vitamin B₁₂*, Science 113 153 Feb. 9 1951.

At the AAAS symposium on "Recent Advances in the Chemistry of the Antibiotics and Vitamins," a chemical method for assay of vitamin B₁₂ was described. The method depends on the liberation by vitamin B₁₂ of one mole of cyanide, a reaction which is accelerated by light. The cyanide is determined by a millimicro method that is sufficiently sensitive to allow the determination of vitamin B₁₂ in urine and tissues.

March & Co., Inc.
Rochester, N. Y.

1103. PRITCHARD H.: *Experiences with the microbiological assay of vitamin B₁₂ in an analytical and consulting laboratory*, Analyst 76 155-160, March 1951.

The tube method of microbiological assay of vitamin B₁₂ with *Lactobacillus leichmannii* S15 has been adapted for routine use in a laboratory where demands for the assay are only intermittent. The vitamin B₁₂ potency has been given for a number of materials used in the compounding trade as determined by this test.

1104. MARSH, M. M., and KUZEL, N. R.: *Separation and determination of crystalline vitamin B₁₂ in synthetic vitamin mixtures*, Anal. Chem. 23: 1773-1776, Dec. 1951.

Substances which interfere in the spectrophotometric determination of vitamin B₁₂ can be satisfactorily removed by mixed cation and anion exchange resin columns.

1105. ROSENBLUM, C., and WOODBURY D. T.: *Determination of the stability of vitamin B₁₂ in multivitamin mixtures by a radioactive indicator method*, J. Am. Pharm. A. (Sc. Ed.) 41 368-371, July 1952.

Authors summary "1. The stability of vitamin B₁₂ in four different multivitamin capsule materials, after storage at room temperature for more than thirteen months, was determined by means of cobalt 60 labeled vitamin as radioactive tracer. 2. The tracer vitamin was extracted by a procedure involving three separate solvents. 3. The quantity of B₁₂ extracted was determined by its radioactivity rather than by conventional chemical assays, and the identification of the radioactivity with the vitamin was made by determining its distribution in the system water-benzyl alcohol. 4. The maximum losses noted ranged between 9 per cent and 31 per cent in more than a year. The valence of the iron (ferrous or ferric) present in the capsule materials had no effect on the stability."

March & Co., Inc.
Rochester, N. Y.

1106. HEINLE, R. W., BETHELL, F. H., CASTLE, W. B., LONDON, I. M., and SALTER, W. T.: *Control of U. S. P. anti-anemia preparations: special report of the United States Pharmacopoeia Anti-anemia Preparations Advisory Board*, J.A.M.A. 151 40-43, Jan. 8, 1953.

Preparations for pernicious anemia are evaluated as of this date. These include vitamin B₁₂, liver extracts, and folic acid. The role of intrinsic factor is discussed.

- 1107 TAUB, A., and LIEBERMAN H.: *Stability of vitamin B₁₂-folic acid parenteral solutions* J. Am. Pharm. A. (Sc. Ed.) 42: 183-186, April 1953.

"The low solubility of folic acid in water except at a relatively high pH, has prevented its use in parenteral solutions in combination with vitamin B₁₂ (cyanocobalamin) which attains optimum stability in the pH range 4 to 6.5. The present study utilizes selected solubilizers for folic acid, including aminoacetic acid, methyl glucamine, and niacinamide within a pH range of 6 to 6.5. Results indicate that niacinamide is the best of the solubilizers studied. By special processing it is possible to dissolve and maintain in solution 5 mg. of folic acid per cc. at pH 6 under conditions compatible with crystal line vitamin B₁₂ at a concentration of 30 µg. per cc. Solutions show no significant loss of potency of either the folic acid or the vitamin B₁₂ after storage at room temperature and under accelerated conditions, and display satisfactory stability with respect to clarity of solutions."

Columbia University College of Pharmacy
New York, N. Y.

1108. CAMPBELL, J. A., McLAUGHLIN J. M., CLARA, J. A., and DUNNETT C. W. *The six point design in the U S P microbiological assay of vitamin B₁₂*. J. Am. Pharm. A. (Sc. Ed.) 42: 276-283, May 1953.

"Since few published data were available concerning the precision of the U.S.P. method for [assaying] Vitamin B₁₂, a detailed statistical study was undertaken to assess the importance of factors affecting the response. On the basis of a linear relation between the logarithm of dose and the logarithm of response, the six point design (three doses of standard and three doses of unknown) was applied to this assay. The design was found to have several advantages, and under well-controlled conditions it may be simplified still further. The method was precise and reproducible. The linear relationship between log dose and log response was valid, and was not influenced by the type of product being assayed, e.g., liver extract injectable or vitamin B₁₂ concentrate. The response to vitamin B₁₂ was influenced by the preparation of tubes and by their position during autoclaving and incubation. To avoid possible bias caused by these factors, the tubes were randomized before addition of the media."

Food and Drug Laboratories
Department of National Health and Welfare
Ottawa, Canada

ANIMAL TISSUES

- 1109 REGISTER, U. D., LEWIS, U. J., THOMPSON H. T., and ELVEHJEM, C. A.: *Variations in the vitamin B₁₂ content of selected samples of pork and beef muscle*, Proc. Soc. Exper. Biol. & Med. 70: 167-168, Jan. 1949

It has been found that vitamin B₁₂ will give a quantitative response to an assay which has been used for a growth factor in liver preparations. This procedure con-

sists of placing rats on a basal diet for a two-week depletion period and then following the growth response during two weeks when the material to be tested is given.

The vitamin B₁₂ activity of pork and beef muscle was tested by this method, using an assayed liver preparation as a standard. The samples of beef were found to contain minimum values of 2 mcg. of vitamin B₁₂ per 100 Gm.; pork from a sow that lactated normally contained 1 mcg. per 100 Gm. and pork from a sow with abnormal lactation (her young died soon after birth) contained only a trace. The wide variation in pork samples may be due to the fact that swine are monogastric animals and therefore may not be supplied with ample quantities of vitamin B₁₂ and possibly other factors that can be produced by microorganisms in the rumen of cattle.

- 1110 REGISTER, U. D., RUEGAMER, W. R., and ELVEHJEM, C. A.: *An improved assay for growth factor in liver extracts*, J. Biol. Chem. 177: 129-134, Jan. 1949

Rats are fed a diet of yellow corn, soybean meal with iodinated casein and vitamins. Injections of liver extract during 1 or 2 weeks give quantitative growth responses. The method proved satisfactory for commercial liver extracts and may also be suitable for the estimation of vitamin B₁₂.

- 1111 FROST D. V., FRICKE, H. H., and SPRUTH, H. C.: *Rat growth assay for vitamin B₁₂*. Proc. Soc. Exper. Biol. & Med. 72: 102-106, Oct. 1949

A method of assaying vitamin B₁₂ by means of rat growth is described. The experimental animals were depleted of vitamin B₁₂ by keeping them on a diet of purified casein for 7 to 14 days (usually 7 since longer depletion increased the mortality). The response of these animals to three vitamin B₁₂ preparations—Cobactone, a concentrate containing 25 mcg. of vitamin B₁₂ per cc., and a concentrate containing any desired concentration of vitamin B₁₂—was then determined. Growth was found to be proportional to the amount of vitamin B₁₂ administered in the critical range of 0.025 to 0.1 mcg. per rat per day. Oral administration at these levels gave approximately the same response as injection. Addition of the vitamin to the diet, multiple dosing, and single dosing on the first day of assay also gave roughly equivalent results. Two liver extracts showed correlation between their effectiveness in rat growth and in their vitamin B₁₂ content, as determined by microbiological assay.

Abbott Research Laboratories
North Chicago, Ill.

1112. SAUBERLICH, H. E.: *The effect of folic acid upon the urinary excretion of the growth factor required by Leuconostoc citrovorum*, J. Biol. Chem. 181: 467-473, Dec. 1949

Urinary excretion of *Leuconostoc citrovorum* factor of rats on a diet un-supplemented by folic acid was low. The urine of rats on a folic acid supplemented diet (1 to 10 mcg./Gm.) was high in citrovorum factor; the activity being proportional to the amount of folic acid ingested. Sulfaguanidine suppressed only slightly the excretion of citrovorum factor; vitamin B₁₂ or ascorbic acid did not increase it. The ingestion of folic acid increased the urinary excretion also in human subj

- 1113 COATES, M. E., HARRISON G. F., and KON S. K.: *The measurement of vitamin B₁₂ by biological assay with chicks* Biochem. J. 46 vii-viii, Feb. 1950 (in Soc. Proc.)

For this assay, newly hatched chicks from hens depleted of vitamin B₁₂ were used. They showed signs of deficiency of vitamin B₁₂ at 4 weeks of age. These depleted chicks responded equally to microbiologically equivalent doses of vitamin B₁₂ in crystalline form, in Examen, and in crude liver extract. Using Examen as the source of vitamin B₁₂, a linear relationship was found at dose levels of vitamin B₁₂ of 1 to 3 mcg./100 Gm. diet between body weight at 4 weeks and the logarithm of the dose. This test proved applicable to the assay of natural products although the limits of error were large. The values obtained for two samples of fish solubles were much higher than those obtained microbiologically by other investigators.

National Institute for Research in Babyhood,
University of London, and
Agricultural Research Council
England

- 1114 TAPPAN D. V., LEWIS, U. J., REGISTER, U. D., and ELVEHJEM, C. A.: *Further studies on the rat growth assay for vitamin B₁₂ activity* Arch. Biochem. 29: 408-412, Dec. 1950.

Authors summary: "The values for vitamin B₁₂ activity obtained by the rat assay method correspond very closely with those obtained using a microbiological procedure. Folic acid has no observable influence on rats receiving the assay ration while cobalt affects the assay only if it is fed at a level high enough to cause a growth depression. Vitamin C exerts an inconsistent but probable growth-promoting effect in the assay. Under the conditions of these experiments autotocytin causes a marked increase in the growth of rats, even in the presence of vitamin B₁₂."

- 1115 BLACK, D. J. G., GETTY, J., COATES, M. E., HARRISON G. F., and KON, S. K.: *Hatchability of eggs and the production of chicks for the assay of vitamin B₁₂* Biochem. J. 46 viii, 1950.

- 1116 COATES, M. E., HARRISON, G. F., and KON S. K.: *The chick assay of vitamin B₁₂ and the animal protein factor* Analyst 76 146-150, March 1951

A method of assay with chicks for vitamin B₁₂ and the animal protein factor is described and its accuracy discussed. Evidence is presented for the existence of other factors besides vitamin B₁₂ in the animal protein factor. Difficulty in obtaining satisfactory responses to crystalline vitamin B₁₂ is reported.

- 1117 SCHWEIGERT B. S., SCHEID H. E., and MARQUETTE, M. M.: *Determination of vitamin B₁₂ in animal tissues*, Federation Proc. 10: 894, March 1951

"Experiments on the effect of adding reducing agents and the method of sterilization of the samples and medium were conducted to improve the method of determining vitamin B₁₂ with *L. leichmannii* as the test organism.

The results obtained when the samples and medium were autoclaved for 10 minutes in the presence of 2 mg. of thioglycolic acid per tube (10 ml. total volume) were comparable to those obtained after Seitz filtration. The activity of several enzyme preparations (trypsin, pancreatin, taka-diastase, chick pancreas and bacterial preparations) was investigated for the release of microbiologically unavailable forms of vitamin B₁₂ in animal tissues. The values obtained for muscle and organ meats were approximately doubled after these treatments as compared to the values obtained for cold water extracts. The vitamin B₁₂ potency of some of these samples was also determined by the rat and chick assay. The range in vitamin B₁₂ potency (expressed as µg./100-gm. fresh weight) determined by the microbiological and animal assays was as follows: beef round, 1.5-2.5; pork loin and ham, 1.0-2.0; lamb leg, 2.0-4.0; beef kidney 15-30 and beef liver 30-100. The vitamin B₁₂ content of the rat livers determined microbiologically was increased as the amount of vitamin B₁₂ fed was increased. The vitamin B₁₂ potency of the meat samples calculated from the data on the vitamin B₁₂ content of the rat livers was in accord with the potency calculated from the growth data."

- 1118 COOPERMAN J. M., and TABENKIN B.: *Vitamin B₁₂ activity of 5,6-dimethylbenzimidazole, 1,2-diamino-4,5-dimethylbenzene and riboflavin for the rat*, Federation Proc. 10: 175, March 1951.

"Rats maintained on the vitamin B₁₂ depletion diets of Register et al. (*J. Biol. Chem.* 177 129 1949) and modified so that 100 mg. crystalline thyroxine per kg. replaced the iodinated casein, and of Emerson (*Proc. Soc. Exper. Biol. & Med.* 70: 892, 1949) were given 5,6-dimethylbenzimidazole (A) 1,2-diamino-4,5-dimethylbenzene (B) and riboflavin (C) to determine the vitamin B₁₂-like activity of these compounds. When administered intraperitoneally compound B had a very low order of activity. Under these conditions compounds A and C can partially satisfy the vitamin B₁₂ requirement. Ten or 15 rats were used in each group. These results are shown in the [accompanying] table.

EFFICIENCY PER RAT	TEST, 2 WING AREA PERCENTAGE at 14	Days of Exposure to diet	Days of Exposure
0			24
0.25 µg. B ₁₂		66	24
0.5 µg. B ₁₂		77	72
1 µg. A	46 (1 dose)		24
1 µg. B	34		24
1 µg. C	42		24
2 µg. C	58		24

Preliminary experiments [suggest] that compound A may be somewhat more effective when given orally, but no increase in effectiveness was noted for compound B when it was administered orally."

Hoffman-La Roche, Inc.
Nutley, N. J.

- 1119 BOXER, G. E., RICKARDS, J. C., ROSENBLUM, C., and WOODBURY, D. T.: *Vitamin B₁₂ and cyanide metabolism*, Federation Proc. 10 166, March 1951

"Methods have been developed which permit the colorimetric determination and isolation, in a form suitable for isotope analysis, of millimicrogram quantities of

cyanide. The following cyanide fractions can be quantitatively separated from urine and tissue homogenates: 1) free (or loosely bound) cyanide, 2) cyanide liberated from vitamin B₁₂ by illumination, 3) cyanide obtained by oxidation of thiocyanate (Boxer and Rickards, *Arch. Biochem. In press*). After injection of 500 μ C¹⁴N (sp. act. 1 mc/mm) into a 9-kg. dog, about 30% of the radioactivity appeared in the urine over a period of 7 days. About half of this activity (15%) was present as thiocyanate representing the established detoxification mechanism for cyanide. The free (or loosely bound) cyanide excreted in the urine during the first 24 hours was of low specific activity indicating at least a 100-fold dilution in a rapidly turning over metabolic pool of cyanide. The cyanide bound in the vitamin B₁₂ excreted in the first 24 hours had a specific activity about 12 times as high as the free cyanide and 4 times as high as the thiocyanate. This indication of a metabolic activity of the cyano group of B₁₂, independent from the rest of the molecule, was further emphasized by the fact that on the following day the specific activity of the B₁₂ cyanide had fallen sharply apparently through interaction with the cyanide pool of low specific activity. A small part of the activity in the urine was found in allantoin.

Smith & Co., Inc.
Rahway, N. J.

1120. STEKOL, J. A., and WEISS, S.: Possible metabolic role of KCN in the rat as studied with C-14-KCN. *Federation Proc.* 10: 252-253, March 1951.

"C-14-KCN was injected intraperitoneally into adult Wistar rats, and 24 hours later the radioactivity was determined in the whole blood, liver and carcass proteins, and in choline (isolated as the reineckate) and creatine (isolated as creatinine-potassium-pyruvate). All of these components contained appreciable radioactivity. Furthermore, C-14-KCN administered together with bromobenzene to rats was utilized for the synthesis of cysteine and acetic acid, as was revealed by the radioactivity located in the cysteine and in the acetic acid of the p-bromophenylmercapturic acid which was isolated from the urine. Aside from the well known metabolic conversion of cyanide to thiocyanate, these observations suggest possible formation and utilization of cyanide *in vivo* either per se or via formaldehyde-formate. Furthermore, the property of cyanides to reduce disulfides to the corresponding thionitriles, and the fact that vitamin B₁₂ contains easily removable cyanide group, point to an interesting possibility of vitamin B₁₂ as the carrier of the metabolically elaborated cyanide with its attendant hematopoietic and other physiological properties."

Lambert Research Institute, and
Institute for Cancer Research
Philadelphia, Pa.

1121. PROCTOR, B. E., and LANG, D. A. *Vitamin B₁₂ content of soft and hard-shell clams*, *Nature* 168: 86-87 July 7 1951. (Letter to Editor)

Fresh samples of soft and hard-shell clams contain more vitamin B₁₂ in mcg./Gm. of dry weight, than pig, beef or calf liver. The visceral mass of the clam contains a higher concentration of vitamin B₁₂ than other parts. This material, which is discarded before clams are minced

for canning, may prove to be an excellent source of vitamin B₁₂ for animal feed supplements.

Massachusetts Institute of Technology
Cambridge, Mass.

1122. COATES, M. E., FORD, J. E., HARRISON, G. F., KON, S. K., PORTER, J. W. G., CUTHBERTSON, W. F. J., and PEGLER, H. F. *Vitamin B₁₂ activity for chicks and different micro-organisms of gut contents and faeces* *Biochem. J.* 49: 1xvii, Oct. 1951. (in Soc. Proc.)

1123. SCHEID, H. E., and SCHWEIGERT, B. S. *Liberation and microbiological assay of vitamin B₁₂ in animal tissues*, *J. Biol. Chem.* 193: 299-305 Nov 1951 (abstr. *J. Am. Dietet. A.* 28: 156, Feb. 1952)

"A series of experiments was conducted with the use of various media and techniques to develop a more uniform assay for determining the vitamin B₁₂ potency of animal tissues. *Lactobacillus leichmannii* 827 was used as the test organism. An amino acid medium that contained 2 mg. thioglycolic acid was found to be satisfactory and the results obtained were comparable to those obtained when aseptic additions or Seitz filtration techniques were used. The vitamin B₁₂ potency of several meat samples was determined. Liver was shown to contain approximately twenty five times and kidney approximately ten to fifteen times as much vitamin B₁₂ activity as pork, beef, or lamb muscle."

1124. FORD, J. E., KON, S. K., and PORTER, J. W. G. *The multiple nature and potency for different micro-organisms of the vitamin B₁₂ activity of calf rumen contents and faeces*, *Biochem. J.* 50: 1x, Dec. 1951. (in Soc. Proc.)

1125. FROST, D. V., FRICKE, H. H., and SPRUTH, H. C.: *Rat growth assay for vitamin B₁₂. Correspondence with collaborative microbiological assay results on U.S.P. liver extracts*, *Federation Proc.* 11: 443-444, March 1952.

"A rat growth assay for vitamin B₁₂ using a highly purified diet with casein has been further studied and improved. Rats used in this study were from the laboratory colony. These animals originally Wistar have been inbred over a period of 20 years. Animals purchased from 1 outside source responded satisfactorily while those purchased from 2 other suppliers did not. It is therefore clear that maternal diet is important in the preparation of weanling rats for this assay. Two liver extract samples, used in 1950 U.S.P. collaborative study of the microbiological vitamin B₁₂ assay were assayed in rats at levels corresponding to average B₁₂ values of 23 partial pating laboratories. The growth responses for the 2 liver extracts at 3 levels in the diet were in close agreement with the response to similar graded levels of crystalline vitamin B₁₂. The method was used to test the specificity of the microbiological method as a measure of the completeness of destruction of vitamin B₁₂ in liver extract by treatment with sodium hydroxide. Results, which were in agreement between rat and microbiological assay indicated that destruction of vitamin B₁₂ was not entirely complete even under conditions of drastic treatment."

antithyroid factor of Ershoff does not appear to complicate this rat growth assay for vitamin B₁₂. Nicotinamide added to the diet depressed the growth of control and supplemented animals, but did not necessarily improve the sensitivity of the method."

Abbott Laboratories
North Chicago, Ill.

1126. DENTON C. A., KELLOGG W. L., and BIRD H. R.: *The apparent vitamin B₁₂ content of eggs as affected by extraction in the presence of cyanide*, Federation Proc. 11 440, March 1952.

"Vitamin B₁₂ was estimated by the microbiological procedure set up by the U. S. Pharmacopoeia. The vitamin was extracted by autoclaving the yolks (whites contain little or no B₁₂) with sodium acetate buffer at pH 4.5 for 30 minutes at 15 pounds, with and without sodium cyanide. When cyanide was not used, yolks from hens on an adequate diet gave an average value of .12 µg. of B₁₂/yolk (range .07-.15). With cyanide present, the value was .48 µg. (range .34-.56). When 1 µg. of B₁₂ was added to each sample the average B₁₂ value obtained was .84 µg./yolk, or a recovery of about 75%. However when cyanide was present during the extraction, an average value of 1.4 µg. was obtained, which was a recovery of 95%. In yolks from hens depleted of B₁₂ the average value without cyanide was 0.2 µg./yolk (range .01-.027). With cyanide, the yolks assayed 1.1 µg. of B₁₂ (range .07-.18). When 1 µg. of B₁₂ was added/yolk, without cyanide present, the average assay value was .81 µg. which represented a recovery of 79%. With cyanide a value of 1.1 µg. was obtained which was about 99% recovery. Similar effects with cyanide have been obtained with tissues and feces as well as with eggs. It appears that the usual methods for the extraction of B₁₂ do not make available to the test organism all the added or naturally occurring B₁₂ of eggs, tissues and feces."

U. S. Department of Agriculture
Beltsville, Md.

FOODSTUFFS AND FEEDSTUFFS

1127. PRODUCTS and PROCESSES *Assay of vitamin B₁₂*, Chem. & Engin. News 27 644 Feb. 23, 1949

"At Food Research Laboratories, Inc., work has been carried out on the assay of vitamin B₁₂ in food and pharmaceutical preparations. The company is accepting samples for the microbiological assay of vitamin B₁₂ activity as well as for the assay of animal protein factor activity by the chick growth method. Results of assays are expressed in terms of crystalline vitamin B₁₂ or if desired, relative to known good sources of these factors, such as liver extracts and fish solubles. Methods are still under investigation for determining the best manner of releasing such bound or conjugated forms of vitamin B₁₂ (or other substitute growth factors) "

1128. PENSACK, J. M., BETHKE, R. M., and KENNARD D. C.: *Some properties of an unidentified growth factor present in fish products*, J. Nutrition 37 353-360, March 1949.

Ohio Agricultural Experiment Station
Wooster, Ohio

1129. RUEGAMER, W. R.: *Occurrence of an unidentified rat growth factor in cottonseed meal*, Arch. Biochem. 23 236-238, Sept. 1949

Cottonseed meal contains a rat growth factor in contrast to soybean meal. It is not certain whether it is identical with vitamin B₁₂ or whether it is some other antithyroid substance. Since considerable heat is applied in processing of the meal, the factor is relatively heat stable.

1130. LEWIS, U. J., REGISTER, U. D., THOMPSON, H. T., and ELVEHJEM, C. A.: *Distribution of vitamin B-12 in natural materials*, Proc. Soc. Exper. Biol. & Med. 72 479-482, Nov 1949

Authors summary: "The vitamin B-12 content of a number of natural materials as determined by the rat assay is presented. Fish solubles, streptomycin slope sheep rumen contents and glandular meats are excellent sources of vitamin B-12. Muscle tissue, eggs and milk products contain lesser amounts, whereas plant materials show no measurable activity."

1131. SCHEID H. E., and SCHWEIGERT B. S.: *Some factors affecting the potencies of vitamin B₁₂ and Lactococcus citrovorum factor of certain natural products*, J. Biol. Chem. 185 1-8, July 1950.

Potencies of vitamin B₁₂ and of the factor required by *Lactococcus citrovorum* were determined in liver extracts, beef liver and beef round, and were found to be very similar when measured with *Lactobacillus leichmannii* 815 and 327 as test organisms. Potency of the *Lactococcus citrovorum* factor and vitamin B₁₂ varied independently. The effect of autoclaving, hot and cold water extraction, alkali treatment, and enzymatic digestion on the potency of vitamin B₁₂ and the *Lactococcus* factor in liver extracts, beef liver and beef round is reported. These studies indicate that complete release of vitamin B₁₂ from natural materials is difficult. Enzymatic techniques or hot water extractions offer considerable promise as a means of releasing bound forms of the vitamin in natural materials.

Anderson Root Institute Foundation, and
University of Chicago
Chicago, Ill.

1132. BICKOFF E. M., LIVINGSTON A. L., and SNELL, N. S.: *The occurrence of vitamin B₁₂ and other growth factors in alfalfa*, Arch. Biochem. 28 242-252, Sept. 1950.

Authors summary: "Microbiological assays for vitamin B₁₂ in alfalfa using *L. leichmannii* as the test organism yielded total apparent vitamin B₁₂ values ranging from 50 to 62 parts per billion (p.p.b.) for fresh alfalfa and from 12 to 45 p.p.b. for dehydrated alfalfa meal. The values obtained varied with sample size, the smaller the sample, the higher the apparent B₁₂ activity."

"Employment of differential assay techniques such as alkali digestion to destroy B₁₂, inclusion of salt in the media which inhibits response to B₁₂, and paper-partition chromatography have indicated that more than 85% of the total apparent vitamin B₁₂ activity is due to factors other than B₁₂. Chick assays indicate that the factors responsible for the apparent B₁₂ activity in microbiological assays do not replace vitamin B₁₂ for chick growth."

"Several techniques, including precipitation of impurities with acetone adsorption of activity on charcoal and chromatography on silica gel have proved successful in concentrating the factors responsible for the apparent B₁₂ activity of alfalfa, but did not preferentially concentrate the pure B₁₂."

Eastern Regional Research Laboratory
Shelton, Conn.

1133. PEELER, H. T., YACOWITZ, H., CARLSON, C. W., MILLER, R. F., NORRIS, L. C., and HEUSER, G. F.: *Studies on the vitamin B₁₂ content of feedstuffs and other materials* J. Nutrition 43: 49-61, Jan. 1951

Authors summary "Evidence is presented that comparable values for vitamin B₁₂ potency are obtained by chick growth and microbiological assays.

"Studies revealed that substances which replace vitamin B₁₂ in the metabolism of *L. leichmannii* can be differentiated equally well with paper partition chromatography or by destruction of the vitamin with alkali.

"Using the rapid microbiological assay method and the alkali correction procedure, a study of the vitamin B₁₂ content of feedstuffs and other materials was made. Fish meal and related products and liver products were found to contain appreciable quantities of vitamin B₁₂, while cereals and their by-products were found to contain very little, if any vitamin B₁₂.

"Two vitamin B₁₂ activity peaks were obtained by paper partition of alfalfa meal extracts. The smaller faster moving peak appeared to be due to thymidine. The other slow moving peak may be due to a form of vitamin B₁₂ not destroyed by alkali, as its R_f value did not correlate with that of any of the known active desoxyribo-sides."

Central Veterinary
Shelton, Conn.

1134. TARR, H. L. A. *Microbiological formation of vitamin B₁₂ in fishery waste materials* Federation Proc. 10: 257, March 1951.

"Since vitamin B₁₂ has been produced microbiologically largely by commercial concerns little information has been made available regarding substrates employed or yields obtained. Though this vitamin occurs abundantly in most fish tissues, especially in the viscera, its distribution in these and in by-products such as fish meals and condensed solubles is very variable [Tarr et al. Food Technology 4: 354, 1950]. With a view to increasing, and possibly standardizing, the vitamin B₁₂ content of such products *Sireptomyces griseus* and *S. aureofaciens* have been cultured with simple aeration in herring press water adjusted to 2% solids content and with no additive other than 2 µg./gm. of cobalt. Microbiological determination of vitamin B₁₂ in the crude fermentation liquids indicated that maximum yields were obtained after about 4 days at 27°. No agreement whatever was obtained in the results of assays of the crude fermentation products with *Lactobacillus leichmannii* in tube turbidimetric procedure and with a cup-plate assay employing a glucose-inorganic salt medium inoculated with a vitamin B₁₂ requiring mutant of *Escherichia coli*. In general the vitamin B₁₂ content as indicated by *L. leichmannii* assay (about 400 µµg./ml) was somewhat similar to that obtained following chromatographic separation on KH₂PO₄ impregnated filter paper development on agar inoculated with the *E. coli* mutant, and subsequent assay of the eluted vitamin. It appears that the hydroxy cobalamine form of vitamin B₁₂ occurs generally in these fermentation products. On treatment with KCN (2.5 mg./ml.) at pH 5.5 about one-half the total activity of the crude products appears as vitamin B₁₂ following chromatographic separation. All activity disappears on treatment with 0.2 N NaOH at 100° for 30 minutes."

Pacific Fisheries Experimental Station
Vancouver, B. C.

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ABSTRACT

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